University of Alberta

The Application of a GC-MS Method to Detect Naphthenic Acids in Natural Waters, Rat Liver, Plasma, and Plant Tissues

by

Mireya del Carmen Merlin Ibarra ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of *Master of Science*

in

Environmental Science

Department of Civil and Environmental Engineering

Edmonton, Alberta

Fall 2007

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-33308-2
Our file Notre référence
ISBN: 978-0-494-33308-2

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

DEDICATION

I would like to dedicate my thesis to Maria del Carmen Ibarra, Juan and Juanito Aguilar for their love and support during my school time.

ABSTRACT

Naphthenic acids are a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids dissolved in the water during bitumen extraction. Naphthenic acids have been traced as the major contributors of toxicity in tailings. Current methods available to detect naphthenic acids in water are based on the detection of the carboxylic acid functional group, thus this methods lack of specificity. This thesis reports the procedure to extract naphthenic acids from water samples, cattail plants, rat liver and plasma that includes extraction, clean up with a strong anion exchange column, and derivatization to form *tert*-butyldimethylsilyl esters of naphthenic acids prior to GC-MS analysis. Reconstructed ion chromatograms for nominal mass of m/z=267 were excellent indicators of the presence of naphthenic acids at concentrations >10 μg L^{-1} for water samples, > 0.5 μg g^{-1} for rat liver, > 0.5 μg mL^{-1} for plasma and > 0.5 μg g^{-1} for cattails.

ACKNOWLEDGEMENTS

This project was possible with the funding provided by Syncrude Canada Ltd., Albian Sands Energy Inc., Suncor Energy Inc., Canadian Natural Resources Limited, True North Energy L.P., and Canadian Water Network. I appreciate the previous work by Joyce Clemente and Fervone Holowenko regarding characterization of naphthenic acids mixtures, and implementation of the GC-MS method that formed the basis for my work. I would like to thank the people from the Chemistry Mass Spectrometry Laboratory at the University of Alberta, Dr Randy Whittal, Don Morgan and Larry Harrower, who helped me with the training and expertise in GC-LRMS analyses and for all the samples analyzed by GC-HRMS. I also acknowledge the people who help collecting water samples and with the analysis of color and TOC: E. Cameron, H. Chen, D. Coy, R. Currah, S. Ebert, F. Kuzmic, J. Manchak and M. Molina for their assistance. I gratefully acknowledge the help of Rozelyn Young with the analysis of naphthenic acids in cattails. Also, Angela Scott, Tara Penner, Jonathan and Nathan for their advice and friendships. I appreciated the collaboration of the M. Wickstrom from the University of Saskatchewan for the rats exposure and Anooshiruan Shayeganpour for provide rat tissues during the method development.

Also, I would like to thank Dr MacKinnon for the water samples and plant tissue provided for this study, and for being open to answer questions and give advice. Finally, my supervisors Dr Selma Guigard for her encouragement to do this project and Dr Fedorak for give me this great opportunity to work with him and for being a great person and supervisor.

TABLE OF CONTENTS

LIST OF TABLES ---

LIST OF FIGURES ---

LIST OF ABBREVIATIONS ---

1. INTRODUCTION --- 1

1.1 Oil Sands Background --- 1

1.2 Naphthenic Acids --- 4
- 1.2.1 Physicochemical Properties of Naphthenic Acids --- 4
- 1.2.2 Naphthenic Acids Sources and Commercial Applications --- 7
- 1.2.3 Naphthenic Acids Toxicity --- 8
  - 1.2.3.1 Aquatic Organisms --- 8
  - 1.2.3.2 Plants --- 10
  - 1.2.3.3 Mammals --- 11
- 1.2.4 Naphthenic Acids Analyses --- 12
  - 1.2.4.1 Methods to Quantify Naphthenic Acids --- 13
  - 1.2.4.2 Mass Spectrometry Methods to Characterize Naphthenic Acids --- 16

1.3 Process-affected Waters by Oil Sands Operations --- 28
- 1.3.1 Surface Waters --- 29
- 1.3.2 Groundwater --- 31

1.4 Objectives --- 34

1.5 Thesis Overview --- 35

1.6 Literature Cited --- 35

2. DETECTION OF NAPHTHENIC ACIDS IN WATER BY GAS CHROMATOGRAPHIC - MASS SPECTROMETRY --- 45

2.1 Introduction --- 45

2.2 Materials and Methods --- 48
- 2.2.1 Naphthenic Acids Mixtures and Chemicals --- 48
- 2.2.2 Choosing Characteristic Ions for RICs --- 48
- 2.2.3 Water Samples --- 49
- 2.2.4 Detection of Naphthenic Acids in Water that Contacted Oil Sands Ore --- 51
- 2.2.5 Extraction of Naphthenic Acids from Water Samples --- 51
- 2.2.6 Solid Phase Extraction Procedure --- 52

2.2.7 Derivation of Extracted Naphthenic Acids ---------- 53
2.2.8 Bromination Procedure ---------- 53
2.2.9 GC-Low Resolution MS (GC-LRMS) Analyses ---------- 54
2.2.10 GC-High Resolution MS (GC-HRMS) Analyses ---------- 54

2.3 Results and Discussion ---------- 55
2.3.1 Choosing Characteristic Ions for RICs ---------- 55
2.3.2 RICs from GC-LRMS Analyses of Merichem Naphthenic Acids ---------- 58
2.3.3 Detection of Naphthenic Acids in Water that Contacted Oil Sands Ore ---------- 60
2.3.4 Sensitivity of the GC-LRMS Method for Naphthenic Acids ---------- 60
2.3.5 Detection of Naphthenic Acids in Water Samples from Various Sources ---------- 62
2.3.6 Addressing the Apparent Anomalous Results from GC-LRMS analyses ---------- 67
2.3.7 Use of SPE Columns to Clean up Naphthenic Acids Fraction ---------- 67
2.3.8 Bromination of Naphthenic Acids-Containing Fractions ---------- 69
2.3.9 GC-HRMS ---------- 70

2.4 Conclusions ---------- 73

2.5 Literature Cited ---------- 75

3. DETECTION OF NAPHTHENIC ACIDS IN PLANTS TISSUES BY GAS CHROMATOGRAPHIC - MASS SPECTROMETRY ---------- 80

3.1 Introduction ---------- 80

3.2 Materials and Methods ---------- 83
3.2.1 Naphthenic Acids Mixtures and Chemicals ---------- 83
3.2.2 Plant Tissue Samples ---------- 83
3.2.3 Plant Tissue Spiked with WIP Naphthenic Acids ---------- 84
3.2.4 Extraction of Naphthenic acids from Plants Tissues ---------- 84
3.2.5 Clean up of Naphthenic acids on Florisil Chromatography Column ---------- 85
3.2.6 Extraction of Naphthenic acids from the Ether Fraction ---------- 85
3.2.7 Analyses of Plant Tissues by GC-LRMS ---------- 86

3.3 Results ---------- 86

3.4 Discussion ---------- 87

3.5 Conclusions ---------- 90

3.6 Literature Cited ---------- 91

4. DETECTION OF NAPHTHENIC ACIDS IN RAT PLASMA AND RAT LIVER BY GAS CHROMATOGRAPHIC - MASS SPECTROMETRY ---------- 95

4.1 Introduction ---------- 95

4.2 Material and Methods--97
4.2.1 Naphthenic Acids Mixtures and Chemicals--97
4.2.2 Biological Samples---98
4.2.3 Rat Plasma Spiked with Merichem Naphthenic Acids------------------------------98
4.2.4 Rat Liver Samples Spiked with Merichem Naphthenic Acids ---------------------99
4.2.5 Rats Exposed to Merichem Naphthenic Acids--99
4.2.6 Extraction of Naphthenic Acids from Rat Plasma-------------------------------- 100
4.2.7 Extraction of Naphthenic Acids from Rat Liver---------------------------------- 101
4.2.8 Analysis of Naphthenic Acids by GC-Low Resolution MS (GC-LRMS) ------ 103
4.2.9 Analysis of Naphthenic Acids by GC-High Resolution MS (GC-HRMS) ----- 104

4.3 Results and Discussion --- 104
4.3.1 Detection of Naphthenic Acids in Rat Plasma Spiked with Merichem Acids-- 105
4.3.2 Detection of Naphthenic Acids in Rat Plasma from Rats fed with Merichem Acids -- 109
4.3.3 Detection of Naphthenic Acids in Rat Liver Spiked with Merichem Acids---- 112
4.3.4 Detection of Naphthenic Acids in Rat Liver samples from Rats Fed with Merichem Acids -- 114
4.5.5 GC-HRMS Analyses of Rat Liver --- 117
4.3.6 Overall Discussion --- 118

4.4 Conclusions --- 122

4.5 Literature Cited --- 123

5. GENERAL DISCUSSION AND SUGGESTIONS FOR FUTURE RESEARCH -- 129

5.1 Discussion -- 129

5.2 Suggestions for Future Research-- 135

5.3 Literature Cited --- 136

APPENDICES -- 140

Appendix A: Extraction of Cattails for Naphthenic Acids Analyses---------------- 140

Appendix B: Settings for Narrow Mass Range Scanning------------------------------ 157

List of Tables

Table 1.1 Expected nominal $[M+57]^+$ masses of *tert*-butyldimethylsilyl derivatives of naphthenic acids that fulfill the given formula $C_nH_{2n+Z}O_2$, based on carbon number (5 to 33) and *Z* families (0 to -12). Adapted from Clemente and Fedorak (2004)....21

Table 2.1 Percentages of ions corresponding to C13 Z =–4 (*m/z*=267), C13 Z=–6 (*m/z*=265) and C14 Z=–6 (*m/z*=279) in 34 different sources of naphthenic acids....50

Table 2.2 Summary of the detection of naphthenic acids from RICs and SIM results in a variety of water samples used in this study....63

Table 2.3 Summary of the detection of naphthenic acids from RICs and SIM results in a variety of water samples used in this study....66

Table 4.1 Summary of the detection of naphthenic acids in rat liver and plasma samples....119

List of Figures

Figure 1.1 General diagram of the Clark Hot Water Extraction Process extraction used in the oil sands industry and cross-section of tailings pond (adapted from MacKinnon 1989). 3

Figure 1.2 Naphthenic acids structures with *Z*=0 to -6 families, R is an alkyl group attached to the ring and m is zero or higher indicating the carboxyl chain length. Adapted from Peng et al. (2002)....6

Figure 1.3 FTIR spectrum of a commercial mixture of naphthenic acids commercial showing the carboxylic acids absorbance peaks at 1743 cm^{-1} for the monomer and 1706 cm^{-1} for the dimer form in dichloromethane. 14

Figure 1.4 HPLC chromatogram of a solution containing 150 mg L^{-1} of Merichem derivatized naphthenic acids, where the hump corresponding to naphthenic acids is integrated from 2.9 to 6 min for quantitative measurements. 15

Figure 1.5 (A) Reaction of a naphthenic acid with the MTBSTFA to form a *tert*-butyldimethylsilyl derivative. (B) Fragmentation pathway of a naphthenic derivative after ionization resulted in the released of a *tert*-butyl group and a major fragment $[M+57]^+$ ion, which is useful in the determination of the parent compounds in the mixture. 19

Figure 1.6 Total ion chromatogram (TIC) of an extracted naphthenic acid mixture from West-In Pit pond at Syncrude site analyzed by GC-MS showing the characteristic unresolved hump in the chromatogram of derivatized naphthenic acids. 19

Figure 1.7 Example of the three-dimensional graphs of extracted naphthenic acids from Syncrude tailings water analyzed by GC-LRMS (A) and by HPLC/QTOF-MS (B), showing the relative intensity versus carbon number and *Z* families taken from Bataineh et al. (2006). 27

Figure 1.8 Tree classification scheme to identify groundwater containing process-affected water from tailings pond, where BCK= background concentrations, PPA = possibly process-affected and PA= process-affected. Adapted from Oiffer (2004)....33

Figure 2.1 Three compounds that would yield *tert*-butyldimethylsilyl esters when reacted with MTBSTFA. Each would give a nominal mass fragments of *m/z* = 267 when analyzed by GC-LRMS, and would be assigned as a naphthenic acid $C_{13}H_{22}O_2$ based on nominal mass. An example of (A) a naphthenic acid, (B) a hypothetical di-unsaturated carboxylic acid, and (C) a hypothetical alcohol. 57

Figure 2.2 (A) TIC chromatogram from the GC-LRMS analysis after extraction of a solution of 100 mg Merichem acids L^{-1}. RICs for (B) *m/z* = 265 (C_{13}, $Z = -6$); (C) *m/z* = 267 (C_{13}, $Z = -4$) and (D) *m/z* = 279 (C_{14}, $Z = -6$). 59

Figure 2.3 (A) TIC chromatogram from the GC-LRMS analysis after extraction of a solution of 10 µg Merichem acids L^{-1} of North Saskatchewan River water. (B) RIC for *m/z* = 267 showing the characteristic hump of naphthenic acids between 15 to 20 min retention time. 62

Figure 2.4 GC-LRMS analyses of the extract of water from an oil storage tank. TIC chromatograms before (A) and after (C) clean up through a SAX SPE column, RICs for *m/z* = 267 before (B) and after (D) clean up through a SAX SPE column. 69

Figure 2.5 GC-MS analyses of the extract of water from domestic well sample 23 (Table 2.3) RICs (*m/z* = 267) before (A) and after (B) bromination, SIM chromatograms (*m/z* = 267.1780) before (C) and after (D) bromination. 73

Figure 3.1 (A) TIC of the extract of 5 g of cattails leaves spiked with 20 µg g-1 of WIP naphthenic acids. RICs for the nominal mass of (B) *m/z*=265, (C) *m/z*= 267 and (D) *m/z*=279 showing a small hump characteristic of the presence of naphthenic acids. 88

Figure 3.2 RIC for the nominal *m/z*= 267 of the extract of 5 g of cattails leaves spiked with 5 µg g^{-1} of WIP naphthenic acids that yield a small hump characteristic of the presence of naphthenic acids. 88

Figure 3.3 (A) TIC of the extract of 5 g of cattails leaves not spiked with WIP naphthenic acids. RICs for the nominal mass of (B) *m/z*=265, (C) *m/z*= 267 and (D) *m/z*=279 do not yield humps with retention time between 15 and 20 min. 89

Figure 4.1 (A) TIC from the extract of 1 mL of rat plasma spiked with 1 μg mL^{-1} of Merichem naphthenic acids; (B) RIC for *m/z* =267 that yield a hump with retention time between 15 and 20 min indicating the presence of naphthenic acids in full mass range scan mode and (C) RIC for *m/z* =267 in narrow mass range scan mode. Peak (x) is from derivatized phosphoric acid used during the initial extraction procedure (A and B) and did not appear when phosphoric acid was replaced with HCl (C) in the extraction method. Resolved peaks of derivatized fatty acids can be observed in the TIC where the most abundant are the *tert*-butyldimethylsilyl esters of hexadecanoic acid (a), 9,12-octadecadienoic acid (b) and octadecanoic acid (c). 107

Figure 4.2 Analysis of the extract of 1 mL of rat plasma spiked with 0.5 μg mL^{-1} of Merichem naphthenic acids, where (A) is the RIC for the *m/z* =267 in full mass range scan mode and (B) is the RIC for the *m/z*=267 acquired in narrow mass range scan mode, when phosphoric acid was replaced with HCl, that yield a hump with retention time between 15 and 20 min indicating the presence of naphthenic acids. Peak (x) is from derivatized phosphoric acid used during the initial extraction procedure. 110

Figure 4.3 Analysis of the extract of 1 mL of rat plasma sample not spiked with naphthenic acids and acidified with HCl. RIC for the nominal mass of *m/z* =267 in narrow mass range scan do not yield a hump that indicated the presence of naphthenic acids. 110

Figure 4.4 (A) RIC for *m/z*=267 that yielded a small hump with retention time between 15 to 20 min indicating the presence of naphthenic acids in 1 mL of plasma sample from naphthenic acids-fed rat #12 and (B) RIC for *m/z*=267 with no hump in the retention time between 15 to 20 min from naphthenic acids-fed rat #13. Both chromatograms were acquired in narrow mass range scan. 111

Figure 4.5 Analysis of the extract of 1 mL of plasma from rat #5 from the control group A that was not fed with naphthenic acids. The RIC for *m/z*=267 was acquired in narrow mass range scan mode with no hump in the retention time window of naphthenic acids. 111

Figure 4.6 (A) TIC of the extract of 5 g of rat liver tissue spiked with 1 μg of Merichem naphthenic acids g^{-1}. (B) RIC for the nominal mass of *m/z*=267 that yielded a hump with retention time between 15 and 20 min characteristic of the presence of naphthenic acids acquired in full mass range scan mode and (C) in narrow mass range scan mode. See Figure 4.1 for identities of a, b and c. 113

Figure 4.7 (A) TIC of the extract of 5 g of rat liver tissue spiked with 0.5 µg of Merichem naphthenic acids g^{-1}. (B) RIC for the nominal m/z=267 yielded a small hump with retention time between 15 to 20 min, acquired in full mass range scan mode. See Figure 4.1 for identities of a, b and c. 113

Figure 4.8 (A) TIC of the extract of 5 g of rat liver tissue not spiked with naphthenic acids. (B) RIC for the nominal m/z=267, which does not yield any hump with retention time between 15 to 20 min, acquired in full mass range scan. See Figure 4.1 for identities of a, b and c. 114

Figure 4.9 (A) TIC of the extract of 5 g of liver tissue from rat #12 fed with 300 mg of Merichem naphthenic acids kg^{-1} and scarified 6 h after ingestion; (B) RIC for the nominal m/z=267 yielding a small hump between 15 to 20 min indicating the presence of naphthenic acids in full mass range scan mode and (C) in narrow mass range scan mode. Peak identities are the same as in Figure 4.1. 116

Figure 4.10 RICs from the extract of 5 g of liver from rat #5 (control group A) not fed naphthenic acids. (A) RIC for the nominal m/z=267 did not yield a hump in the retention time window of naphthenic acids in full mass range scan mode and (B) in narrow mass range scan mode. 116

Figure 4.11 SIM for the ion with exact mass of 267.1780 of the extract of 5 g of rat liver sample from rat # 16 fed with 300 mg Merichem naphthenic acids kg^{-1} body weight and scarified 6 h after oral ingestion 118

List of Abbreviations

ETB	East Toe Bern
FTIR	Fourier transform infrared spectroscopy
GC-LRMS	Gas chromatography with low-resolution mass spectrometry
GC-HRMS	Gas chromatography with high-resolution mass spectrometry
HPLC	High performance liquid chromatography
MTBSTFA	*N*-methyl-*N*-(*t*-butyldimethylsilyl) trifluoracetamide
RIC	Reconstructed ion chromatogram
SIM	Single ion monitoring
TAN	Total acid number
TIC	Total ion chromatogram
WIP	West-In Pit tailings pond

1. Introduction

1.1 Oil Sands Background

One of the largest reserves of petroleum in the world contains approximately 1.75-2.5 trillions barrels of crude oil (Lo et al. 2006), of which 200 millions barrels are mineable bitumen with current technology (Masliyah 2002). These deposits are located in three geographic areas: the Athabasca, the Peace River and the Cold Lake areas in the Province of Alberta, Canada. All three deposits are suitable for oil production, but the Athabasca oil sands contain about 80% of the total and are the only suitable deposit for surface mining (Chastko 2004).

The commercial production of the Athabasca oil sands began in 1967, when the first mine operated by the Great Canadian Oil Sands Company started mining. Today, the Athabasca oil sands have three main active mines: Syncrude Canada Ltd., Albian Sands Energy Inc., and Suncor Energy Inc. These mines are located adjacent to the Athabasca River or its tributaries. With the increasing demand for oil and the continuing development of recovery technology, several other mines are in different stages of development (Alberta Department of Energy 2005).

The Alberta oil sands are composed of bitumen (~20%), water (~5%), sand, silts and clays (75%), and other particulate materials (~1%). The bitumen in the Athabasca deposits is mined from the oil sands by using an open-pit technique, whereas conventional oils are extracted by drilling wells. The recovered bitumen is a heavy, black and viscous material similar to molasses at room temperature and is composed of naphthenic acids, alkanes, aromatics, resins and asphaltenes (Kasperski 1992, Yang and

Czarnecki 2005). The bitumen is separated from the sand by using hot water extraction. In 1932, Dr. Karl Clark developed this technology (Clark and Pasternak 1932), which is currently being used by the three main active mines at Athabasca. When the Clark Hot Water Extraction Process is used, the mined ore is mixed with alkaline hot water and air, thus the bitumen floats to the surface as primary froth, and the material remaining (slurry of water) is concentrated in the tailings (MacKinnon 1989, Schramm et al. 2000). Figure 1.1 shows a general diagram of the Clark Hot Water Extraction Process and a cross-section of the tailings pond.

The tailings are the residual material remaining after bitumen extraction and are slurry of sand, fine minerals, clay, water, and unrecovered bitumen in a stable aqueous suspension (MacKinnon 1989). This suspension is characterized by an alkaline pH between 8 and 9 (Kasperski 1992), high concentrations of dissolved naphthenic acids and high salinity (Lo et al. 2003). Environmental regulations in the province prevent the oil sands companies from discharging any fluid waste resulting from the bitumen extraction to the environment due to concerns about toxicity (Lo et al. 2003). Thus, the tailings are stored in large surface ponds for future reclamation (Rogers et al. 2002a).

When the slurry of water, resulting from extraction of the ore, is pumped to the tailings ponds, the solids separate. As shown in Figure 1.1, tailings deposit showed a stratified cross-section in the tailings pond. Coarse sands settle quickly and this sand is used to build dykes and beaches around the ponds. Fine minerals form a stable suspension and clarified water from the tailings ponds is recycled for use in ore processing (Schramm et al. 2000). In recent years, gypsum has been used to facilitate the settling of fine suspended materials producing consolidated or composite tailings and clarified water (Crowe et al. 2001).

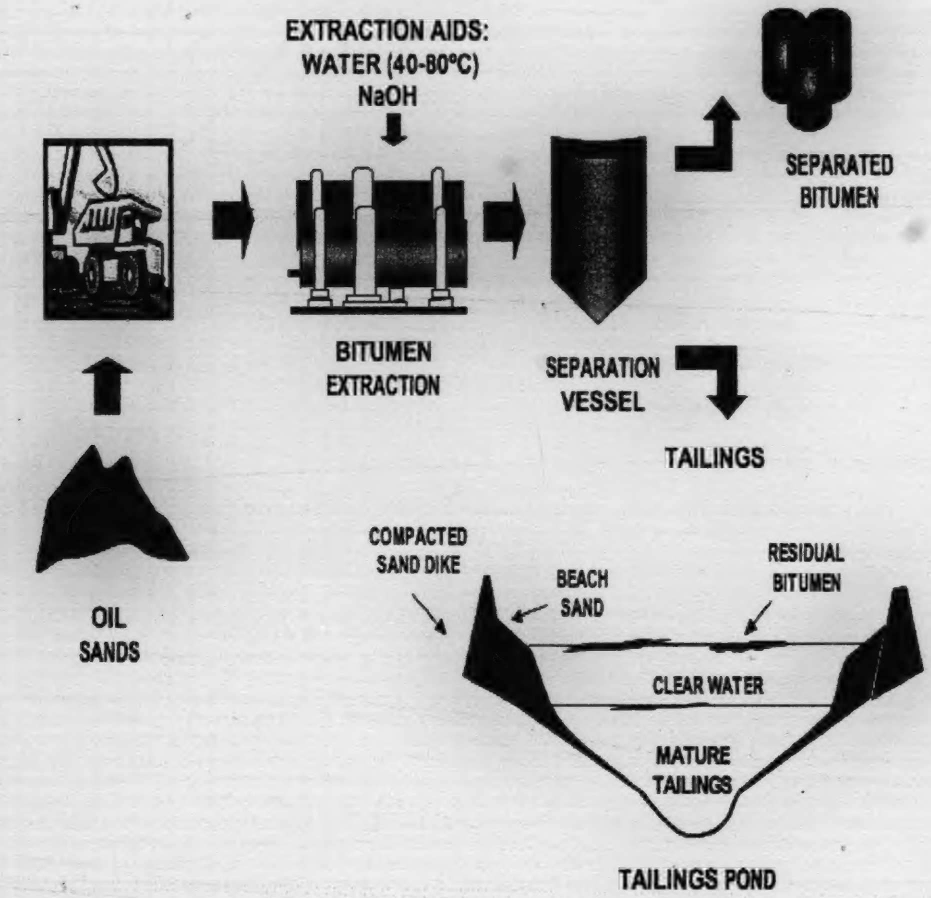

EXTRACTION AIDS:
WATER (40-80°C)
NaOH
SEPARATED
BITUMEN
BITUMEN
EXTRACTION
SEPARATION
VESSEL
TAILINGS
OIL
SANDS
COMPACTED
SAND DIKE
BEACH
SAND
RESIDUAL
BITUMEN
CLEAR WATER
MATURE
TAILINGS
TAILINGS POND

Figure 1.1 General diagram of the Clark Hot Water Extraction Process extraction used in the oil sands industry and cross-section of tailings pond (adapted from MacKinnon 1989).

Environmental concerns about the tailings waters are the presence of naphthenic acids. These acids are the principal contributors of toxicity to aquatic species, some plants, and animals (CONRAD 1998). Naphthenic acids are dissolved as sodium naphthenates, during the bitumen extraction, which are routinely extracted under alkaline conditions and are concentrated in the tailings (Herman et al. 1994). Concentrations of naphthenic acids in tailings waters range from 20 to 120 mg L^{-1} (Holowenko et al. 2001, Yen et al. 2004). These concentrations are related to the age and activity of the pond. Ponds receiving fresh input of tailings have concentrations > 100 mg L^{-1}, and ponds not receiving tailings for 7 or 11 years have concentrations about 20 mg L^{-1} (Clemente and Fedorak 2004). The decreasing concentration is the result of natural degradation by indigenous bacteria (Clemente and Fedorak 2005, Scott et al. 2005).

1.2 Naphthenic Acids

Naphthenic acids are mixtures of monobasic carboxylic acids found in almost all petroleum sources (Brient et al. 1995), including the Athabasca bitumen, and these acids are the result of partial biodegradation of petroleum by microorganisms (Tissot and Welte 1978). These petroleum acids are dissolved into the alkaline aqueous phase during the hot water extraction, and are the principal contributor of toxicity in the tailings (CONRAD 1998).

1.2.1 Physicochemical Properties of Naphthenic Acids

Naphthenic acids are a complex mixture of alkyl-substituted acyclic aliphatic and cycloaliphatic carboxylic acids and are defined by the general formula $C_nH_{2n+Z}O_2$, where n indicates the number of carbon atoms, $2n+Z$ represents the number of hydrogen atoms and Z is zero or a negative even integer indicating the number of hydrogen atoms lost during the ring formation (Holowenko and Fedorak 2001, Clemente et al. 2003a). Previous studies showed that typical values for n vary from 5 to 33 (Holowenko et al. 2001, Lo et al. 2003, Clemente and Fedorak 2004, Frank et al. 2006). However, the findings of Bataineh et al. (2006) indicate that carbon numbers beyond 20 in any Z-series are absent from naphthenic acids mixtures. Their detection in previous studies was the result of double derivatization due to isomer hydroxylation as postulated by Clemente et al. 2004 and the low accuracy of mass spectrometry methods based on unit mass results. Thus, in the newly develop method n vary from 9 to 20. (See Section 1.2.4.2).

Figure 1.2 shows the general structures of naphthenic acids. In this figure, m is zero or higher, R is an alkyl group in the cyclic structure, and compounds with Z=0 correspond to saturated acyclic acids. In the case of naphthenic acids, these acyclic compounds are more likely to be highly branched than linear fatty acids (Rudzinksi et al. 2002), although fatty acids fit the general formula for naphthenic acids. The simplest cyclic structures of naphthenic acids with Z=-2 are monocyclic carboxylic acids with either a cyclohexyl or a cyclopentyl ring. As values of Z decrease from -2 to -4 to -6 and so on, the cyclic structures become more complex with multiple combinations of saturated rings and aliphatic side chain distribution (Tomczyk et al. 2001). There is also considerable diversity in the number of rings, which are fused or bridged (Brient et al. 1995). As a result of this complexity, individual isomers of the naphthenic acids mixture have not been isolated (Holowenko et al. 2002).

Naphthenic acids purified mixtures are pale yellow viscous liquids, with a characteristic odor due to the phenol and sulfur impurities. Naphthenic acids are soluble in most organic solvents, and in aqueous solutions at alkaline pH. These acids behave as characteristic carboxylic acids with dissociation constants (Ka) from 10^{-5} to 10^{-6} (Brient et al. 1995). Naphthenic acids are non-volatile, chemically stable, and amphiphilic compounds involved in the stabilization of emulsions (Saab et al. 2005).

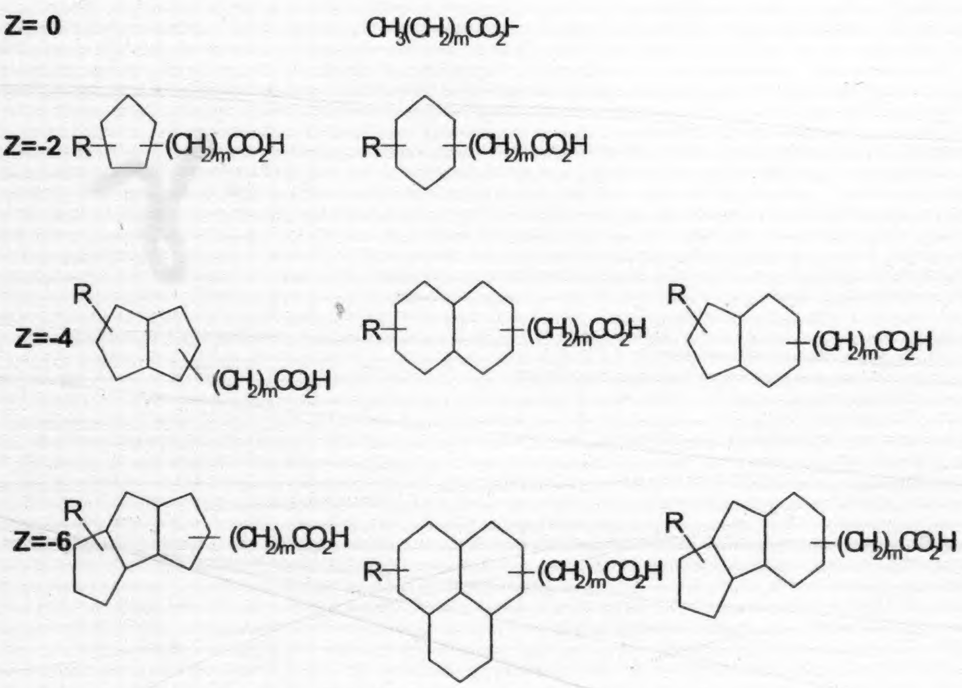

Z= 0
CH3(CH2)mCO2H
Z=-2
R
(CH2)mCO2H
R
(CH2)mCO2H
Z=-4
R
(CH2)mCO2H
R
(CH2)mCO2H
R
(CH2)mCO2H
Z=-6
R
(CH2)mCO2H
R
(CH2)mCO2H
R
(CH2)mCO2H

Figure 1.2 Naphthenic acids structures with *Z*=0 to -6 families, R is an alkyl group attached to the ring and m is zero or higher indicating the carboxyl chain length. Adapted from Peng et al. (2002).

1.2.2 Naphthenic Acids Sources and Commercial Applications

Small amounts of naphthenic acids are found in petroleum sources around the world, generally at concentrations less than 3% by weight (Slavcheva et al. 1999). Their presence in petroleum deposits is the result of two processes: Originating during the petroleum formation and resulting from partial biodegradation of oil deposits by the microorganisms (Saab et al. 2005). Consequently, each petroleum deposit has a unique mixture of naphthenic acids from which, the reservoirs maturity and degree of biodegradation can be estimated (Nascimento et al. 1999).

Commercial preparations of naphthenic acids with different properties are available for industrial purposes. These mixtures are extracted from petroleum distillates. In this extraction, the distillate fractions of diesel and kerosene are washed with 2% to 10% solutions of NaOH at temperatures between 200 and 370°C are isolated (Brient et al. 1995). Then, the refined mixtures of naphthenic acids are marketed by physical properties such as color, impurities or total acid number (TAN), which is the amount of KOH required to neutralize the acidity of 1 g of oil (Slavcheva et al. 1999). Naphthenic acids are combined with metals, amides or esters to produce different derivatives, which are used for a wide variety of industrial products including wood preservatives (Barnes et al. 2005), plasticizers and lubricants (Mustafaev and Shikhalizade 1980), solvents for the extraction of rare-earth metals (du Preez and Preston 1992), emulsifiers, defoamers, tire cord adhesives, vinyl stabilizers, paint or ink driers among others (Brient et al. 1995). Refined naphthenic acids mixtures used in commercial products are more degradable than oil sands naphthenic acids (Scott et al. 2005), and widespread use of commercial mixtures represent a concern, because they are an additional source for naphthenic acids to enter aquatic ecosystems.

1.2.3 Naphthenic Acids Toxicity

Previous studies (MacKinnon and Boerger 1986, CONRAD 1998, Rogers et al. 2002a) have identified naphthenic acids as the major toxic compounds in the oil sands tailings waters. Because individual components of these mixtures have not been isolated, the exact mode of toxicity of each compound has not been determined. Naphthenic acids are surfactants and they can damage membranes (Schramm et al. 2000). Aquatic species seem to be the most affected, because they are in constant contact with naphthenic acids in their habitats, the naphthenic acid resulting from either commercial activities or petroleum extraction activities. Recent studies have also shown that aspen seedlings exposed to 300 mg of commercial naphthenic acids L^{-1} (Kamaluddin and Zwiazek 2002) and Wistar rats exposed to 300 mg of purified oil sands naphthenic acids kg^{-1} body weight (Rogers et al. 2002a) were adversely affected by petroleum acids at those concentrations.

1.2.3.1 Aquatic Organisms

Naphthenic acids are dissolved as sodium naphthenates in the oil sands effluents and numerous studies have demonstrated that they are responsible for the toxicity to aquatic species (CONRAD 1998). For example, Dokholyan and Magomedov (1983) conducted an aquatic toxicity study with Caspian fish species exposed to sodium naphthenates solution in acute and chronic experiments. They produced 96-h LC_{50} values of 25 mg of naphthenic acids L^{-1} for young chum salmon (*Oncorhynchus keta*), 50 mg of naphthenic acids L^{-1} for adult kutum (*Rutilus frisii kutum*), roach fingerlings (*Rutilus rutilus caspicus*) and sturgeon (*Acipenser gueldenstaedi*), and 75 mg of naphthenic acids L^{-1} for adult Caspian round goby (*Negobius melanostomus affinis*). Additionally, chronic toxic effects at low concentrations showed that the young chum salmon was the most affected species with a lethal dose of 3 mg of naphthenic acids L^{-1} after 60 d of exposure. The results from acute and chronic exposure test indicate that the toxic effects are dependent on the age and sensitivity of the species studied. Subsequently, Dokholyan and Magomedov (1983) measured other parameters of response in addition to mortality to sodium naphthenates such as erythrocyte and leucocyte count, hemoglobin content, blood sugar and glycogen content in the tissues. Nonlethal effects were observed including decreased in blood glucose by 50% after 5 d, reduced leucocyte count by 15 to 35% after 10 d and increased muscle glycogen. Based on the results, Dokholyan and Magomedov (1983) established threshold values of sodium naphthenates: 0.5 mg L^{-1} for biochemical effects and 0.15 mg L^{-1} as the maximum permissible concentration of naphthenic acids in seawater. These values are not viable for most of the ponds, lakes or rivers at the Athabasca region, which have concentrations over 0.15 mg L^{-1} (Leung et al. 2003). In addition, Dokholyan and Magomedov (1983) emphasize that the chemical composition of the naphthenic acids mixture plays an important role in toxicity, so these results should be cautiously extrapolated to other sources of naphthenic acids.

Another toxicity study showed that the acute lethality of oil sand tailings from Mildred Lake Settling Basin (holding pond at Syncrude site) to rainbow trout (*Onchorhynchus mykiss*) and water fleas (*Dophnia magna*) was significantly reduced when the oil sands effluent was treated to remove naphthenic acids using an acid extraction technique (MacKinnon and Boerger 1986). Similarly, Peters (1999) measured the incidence of mortality and deformity of Japanese medaka embryos incubated in oil sands process-affected waters from various experimental ponds on Syncrude Canada's site that contain naphthenic acids. The main toxic effects included decreased embryo survival, hatching success, increased in incubation time prior to hatching and increased occurrence of deformity.

Recent studies measured the ecological effects of naphthenic acids and major ions (dominated by sodium, sulfate and chloride) from various process-affected waters on phytoplankton communities. Changes in taxonomic composition were associated with the presence of naphthenic acids at concentrations > 40 mg L^{-1} and major ions concentrations indexed by conductivity, where Chlorophyta was the dominant community. Also, six different algal divisions were identified as tolerant to naphthenic acids with little ecological effects at concentrations of 6.5 mg L^{-1} (Leung et al. 2003).

1.2.3.2 Plants

The toxic effects of naphthenic acids on plants have been difficult to assess as other components in the tailings waters such as high concentration of ions (Na^+, F^-, and SO_4^{2-}), and high alkaline pH (8.5 to 10.15) also exert similar toxic effects on plants (see review by Renault et al. 1999). In microcosms studies, Bendell-Young et al. (2000) noted that cattail plants exposed to oil sands effluents under controlled environments increased their photosynthetic rates in comparison to cattails growing in non-impacted waters. In a similar study, Crowe et al. (2001) studied the effects of oil sands effluents on cattails (*Typha latifolia* L.) and alsike clover plants (*Trifolium hybridum* L.) growing in natural wetlands on Suncor's site, which are characterized by high ionic content and naphthenic acids. Cattails and clover plants exposed to oil sands waters showed higher levels of apparent photosynthesis than control plants growing in non-impacted sites. In addition, Crowe et al. (2001) found that plants growing in impacted wetlands were well adapted, but the long-term survival of the plants was not determined and is necessary in order to ensure the viability of the wetland.

A more specific study conducted by Kamaluddin and Zwiazek (2002) found that naphthenic acids inhibited aspen (*Populous tremuloides* Michx.) seedlings. They treated seedlings with 75 mg, 150 mg and 300 mg of commercial naphthenic acids L^{-1} and these seedlings showed a decrease in leaf chlorophyll concentrations, root water flow, root respiration, leaf stomatal conductance, photosynthesis, chlorophyll concentration, and leaf size. Furthermore, Kamaluddin and Zwiazek (2002) measure the uptake of naphthenic acids by the roots in xylem exudates of aspen seedlings by a Fourier transform infrared (FTIR) spectroscopy method. Naphthenic acids were detected in concentrations of about 15 mg L^{-1} for aspen seedlings treated with 150 mg L^{-1} and 21 mg L^{-1} for aspen seedlings treated with 300 mg L^{-1}. The results indicate that naphthenic acids can be can be taken up and transported by the roots of aspen seedlings, affecting the uptake of essential nutrients and ultimately the metabolism of the plants (Kamaluddin and Zwiazek, 2002).

1.2.3.3 Mammals

Mammalian toxicity to naphthenic acids has not been as extensively studied as aquatic toxicity, but a recent study conducted by Rogers et al. (2002a) characterized mammalian toxicity using Wistar rats. In this study, male and female rats with similar characteristics were used in acute and chronic experiments. In the acute study, the rats were fed a single oral dose of 3, 30 or 300 mg of naphthenic acids kg^{-1}. These doses correspond to 0.5, 5 or 50 times the worst-case scenario of the dose received in natural conditions at the Athabasca oil sands. After 14 d of exposure, the rats were sacrificed, the organs removed and the toxic effects were measured based on organ damage and change in organ weights as compared to control animals. The most evident effects were observed in rats fed with the highest dose (300 mg kg^{-1}) and some differences were also observed between genders. For example, female rats showed an increase in ovary and spleen size, whereas male rats showed an increase in heart and testes weight. In addition, female rats manifested more occurrence of liver damage, while males rats showed more occurrence of brain hemorrhage. In the chronic study, only female rats were orally exposed to naphthenic acids extracted from tailings at concentrations of 0.6, 6 or 60 mg kg^{-1}, for 5 d per week for 90 d. After 90 d of exposure, the rats receiving the highest dose showed accumulation of glycogen and severe damage to the liver. Kidneys and brain were also affected by ingestion of naphthenic acids and this resulted in increased weight and necrosis. The findings of Rogers et al. (2002a) indicate that the liver was the target organ of naphthenic acids in both treatments, and the toxic effects were dependent of different factors including the concentration of naphthenic acids, frequency of exposure, and gender.

1.2.4 Naphthenic Acids Analyses

Naphthenic acids concentration in petroleum sources is measured by titration with potassium hydroxide for TAN. This measurement is not specific to determine naphthenic acids concentration because other acidic compounds present in petroleum are neutralized in addition to naphthenic acids and account for TAN (Nascimento et al. 1999).

Currently, the two methods available to determine naphthenic acid concentrations in aqueous solutions are based on the detection of carboxylic acids as a functional group. Examples of these methods are a FTIR spectroscopy procedure that is commonly used by the oil sands industry and a high performance liquid chromatography (HPLC) method. These two methods yield comparable results, but they are not specific to naphthenic acids because in essence any carboxylic acid is quantified as a naphthenic acid. Many mass spectrometry methods have been developed to characterize naphthenic acids mixtures, but most of these methods are highly specialized and require costly equipment and specific training not always available in conventional laboratories. Mass spectrometry methods have provided important information about naphthenic acids structures and composition. To date, regardless of the method of analysis used, the separation or quantification of individual isomers of naphthenic acids have not been achieved. Some of these methods are reviewed in detail in the following sections.

1.2.4.1 Methods to Quantify Naphthenic Acids

At present, there is no analytical method that quantifies individual isomers of naphthenic acids, however total naphthenates in aqueous solutions can be determine by HPLC or FTIR spectroscopy. A quantitative analysis of naphthenic acids by a FTIR spectroscopic method (Jivraj et al. 1995) is based on the absorbance of the carbonyl group of the carboxylic acid at wavenumbers of 1743 cm^{-1} for the monomer form and 1706 cm^{-1} for the dimer form in the IR spectrum of the extracted carboxylic acids in dichloromethane. The FTIR method involves the extraction of the carboxylic acids from acidified aqueous samples into dichloromethane, concentration of the extracts and further analysis. An example of the naphthenic acids spectrum is given in Figure 1.3 showing the characteristic peaks at 1706 cm^{-1} and 1743 cm^{-1} of a commercial mixture of naphthenic acids and the response of the reagent blanks with no naphthenic acids.

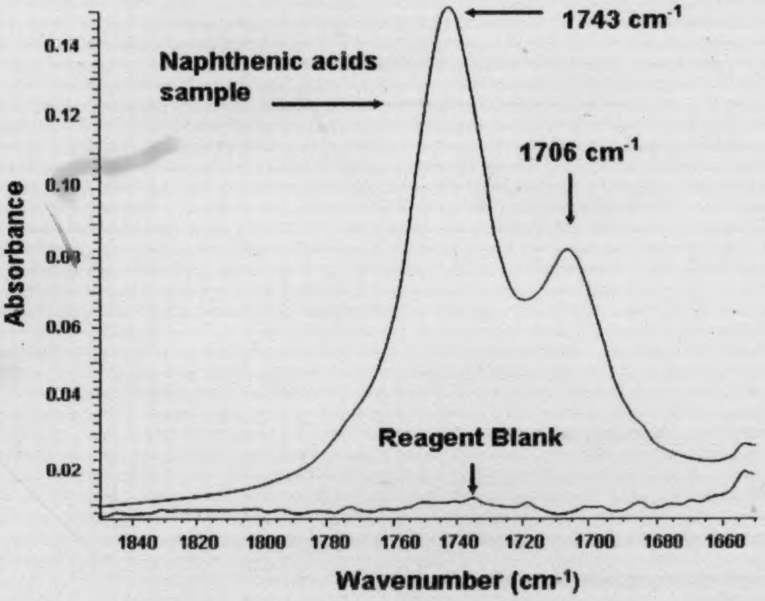


Figure 1.3 FTIR spectrum of a commercial mixture of naphthenic acids commercial showing the carboxylic acids absorbance peaks at 1743 cm^{-1} for the monomer and 1706 cm^{-1} for the dimer form in dichloromethane.

Naphthenic acids extracted from the oil sands have almost identical FTIR spectrum as naphthenic acids extracted from commercial mixtures (Rogers et al. 2002b). The sum of monomer and dimer peak absorbances of an unknown sample can be interpolated in a standard calibration curve prepared at concentrations of 1 to 300 mg kg^{-1} in dichloromethane using Kodak acids, a commercial mixture of naphthenic acids, to determine the concentration of naphthenic acids in a sample (Jivraj et al. 1995).

Although, the FTIR method requires a laborious extraction procedure, this method is the most commonly used method to quantify naphthenic acids in tailings pond waters.

Clemente et al. (2003b) derivatized the carboxyl group of naphthenic acids with 2-nitrophenylhydrazine and the derivatized carboxylic acids were analyzed by HPLC with the detector set at 400 nm. Because naphthenic acids are complex mixtures of carboxylic acids, the derivatized compounds elute from the column as a unresolved hump that is integrated from 2.9 to 6 min. An example of a HPLC chromatogram of Merichem commercial naphthenic acids at a concentration of 150 mg L^{-1} is given in Figure 1.4, showing the peaks corresponding to the unreacted derivatizing reagents that eluted within 2.9 min and the integrated naphthenic acid hump from 2.9 to 6 min.

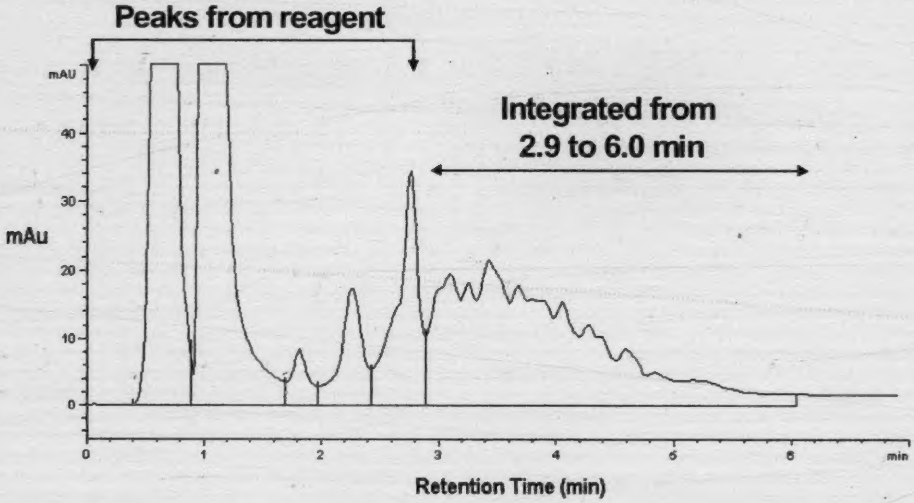


Figure 1.4 HPLC chromatogram of a solution containing 150 mg L^{-1} of Merichem derivatized naphthenic acids, where the hump corresponding to naphthenic acids is integrated from 2.9 to 6 min for quantitative measurements.

Similar to the FTIR method, the concentration of an unknown sample is estimated by interpolation of the area under the hump of derivatized commercial naphthenic acids mixtures used to prepare a calibration curve. Unlike the FTIR method, the HPLC method does not require extraction, because it uses a small sample volume with little sample preparation. However, oil sands naphthenic acids and commercial naphthenic acids show different hump shapes in the HPLC chromatograms, thus the area of an unknown sample must be interpolated with the standard curve that most resemble the analyzed mixture in order to have similar results as with FTIR (Scott et al. 2005). Yen et al. (2004) modified the HPLC method to detect concentrations as low as 5 mg L^{-1} and compared the FTIR and HPLC methods by analyzing 58 aqueous samples from the Athabasca oil sands. Yen et al. (2004) demonstrated that the HPLC method was less sensitive than the FTIR method. In general, the concentrations obtained from the FTIR method were 11% higher than those from the HPLC method.

Currently, FTIR spectroscopy and HPLC are used to measure concentrations of naphthenic acids in water samples, but both methods lack specificity because these methods base the analyses on detecting carboxylic acids and do not have the ability to resolve individual naphthenic acids. Thus, naturally occurring fatty acids present in river waters or in any organism are measured as naphthenic acids with either method.

1.2.4.2 Mass Spectrometry Methods to Characterize Naphthenic Acids

A variety of mass spectrometry methods exist to characterize complex mixtures of naphthenic acids. Some common methods include gas chromatography-electron impact mass spectrometry (GC-MS), negative ion mass spectrometry techniques such as fast atom bombardment mass spectrometry (FABMS) or fluoride negative ion chemical ionization MS, electrospray ionization mass spectrometry (ESI-MS), atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and reverse phase capillary HPLC with quadrupole time of flight mass spectrometry detector (QTOF-MS) among others. None of these methods is able to resolve the naphthenic acid mixture into individual components; however, all of them have provided qualitative data useful to characterize naphthenic acids mixtures. This section will discuss the results of previous studies that have used mass spectrometry methods to investigate the nature of naphthenic acids mixtures.

Siefert and Teeter (1969) studied the carboxylic acids in California crude oils using a combination of thin layer chromatography (TLC) and mass spectrometric techniques. Siefert and Teeter (1969) observed structural diversity in the acids extracted, with more than 1500 compounds detected. Naphthenic acids were derivatized to their 1,1,7-trihydroperfluoroheptyl esters to simplify the interpretation of the mass spectrum. Isolated fractions of California crude have also been characterized by fluorine negative ion chemical ionization MS (NICI-MS) to produce ring type and carbon number. A diverse group of naphthenic acids were reported with 0 to 6 rings and 10 to 35 carbon numbers (Dzidic et al. 1988). Using negative ion FAB mass spectrometry, Fan et al. (1991) analyzed the acid fractions isolated from California, Montana and Louisiana crudes. The results of Fan et al. (1991) showed a wide range of naphthenic acids with carbon numbers from 10 to 50 and with 0 to 6 rings. Using fast ion bombardment mass spectrometry (FIBMS) in negative ion mode, Wong et al. (1996) characterized the organic acids from a bed of activated carbon used for wastewater a treatment at petroleum refinery. Naphthenic acids were recovered from the carbon bed with a modified supercritical fluid extraction (SFE) procedure and were characterized based on their molecular weight distribution. Mass spectra data from commercially available Kodak naphthenic acids gave similar results to the adsorbed carboxylic acids, indicating the presence of naphthenic acids in refinery wastewater.

Several GC-MS methods have been developed to further study the complex mixture of naphthenic acids. Green et al. (1994) applied a GC-MS method to evaluate the suitability of esterification with fluoroalcohols. In this procedure, the hydroxyl group and the carboxyl group were simultaneously converted to trifluoroacetate ester and fluoroalcohol esters, which resulted in improved volatility and GC-MS behavior. This procedure was applied to petroleum distillates from Texas Refinery and the results showed a wide range of acyclic isoprenoids acids and a hump of unresolved naphthenic acids that could not be interpreted due to its complexity and to insufficient mass resolution power.

Later, St. John et al. (1998) applied a GC-MS method to characterize two unidentified commercial naphthenic acids mixtures. In the method of St. John et al. (1998), naphthenic acids were derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to their *tert*-butyldimethylsilyl esters, which produce stable fragments after the electron impact ionization and yield characteristic [naphthenate + dimethylsilyl]$^+$ ions (Figure 1.5), frequently referred as [M+57]$^+$ ions, where M represents the molecular mass of the underivatized naphthenic acid. After derivatization, the *tert*-butyldimethylsilyl esters of naphthenic acids elute from the GC column as unresolved hump, as shown in Figure 1.6.

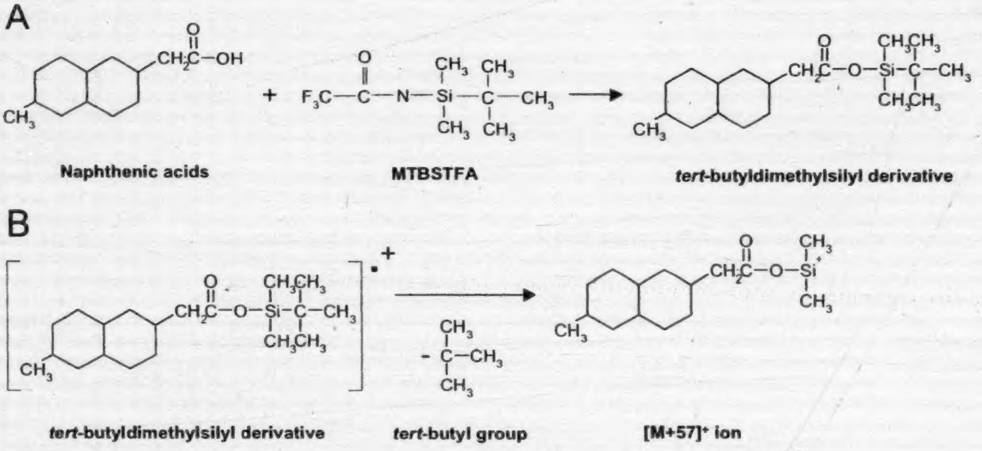

A
Naphthenic acids
MTBSTFA
tert-butyldimethylsilyl derivative
B
tert-butyldimethylsilyl derivative
tert-butyl group
[M+57]+ ion

Figure 1.5 (A) Reaction of a naphthenic acid with the MTBSTFA to form a *tert*-butyldimethylsilyl derivative. (B) Fragmentation pathway of a naphthenic derivative after ionization resulted in the released of a *tert*-butyl group and a major fragment $[M+57]^+$ ion, which is useful in the determination of the parent compounds in the mixture.

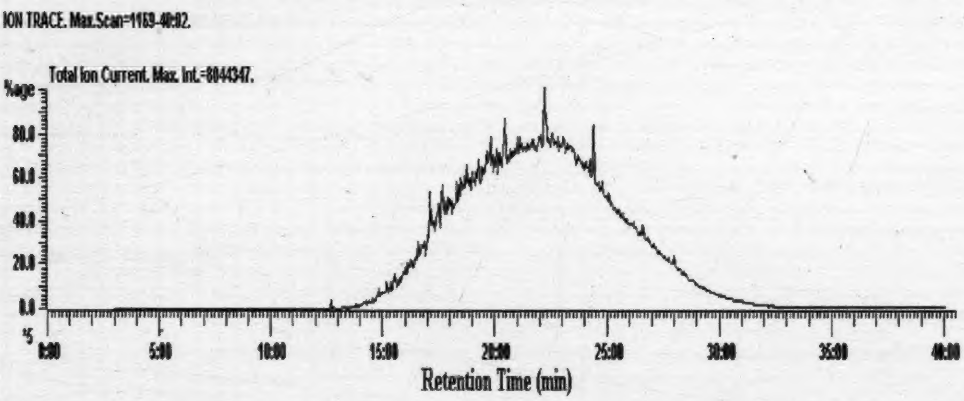


Figure 1.6 Total ion chromatogram (TIC) of an extracted naphthenic acid mixture from West-In Pit pond at Syncrude site analyzed by GC-MS showing the characteristic unresolved hump in the chromatogram of derivatized naphthenic acids.

St. John et al. (1998) classified individual components eluting within the unresolved hump of naphthenic acids by carbon numbers and *Z* families. Their results were presented in a table with 197 empirical formulae of naphthenic acids and molecular masses that correspond to each formula. A similar matrix is shown in Table 1.1 with the corrections made by Holowenko et al. (2002). Each of the 156 formulae in Table 1.1 represents at least one structure and the given nominal masses correspond to $[M+57]^+$ ion. Holowenko et al. (2002) applied the method of St. John et al. (1998) to characterize naphthenic acids, in oil sands ores, in commercial mixtures and in process-affected waters produced by the oil sands industry. Holowenko et al. (2002) presented their data in three-dimensional plots as percentage composition of naphthenic acids based on carbon numbers and *Z* families. An example of the three-dimensional plot is provide in Figure 1.7A. Each plot was normalized to the molecular signal of highest intensity ion in the mass range selected and the resulting three-dimensional bar graphs clearly showed the differences between naphthenic acids from oil sands tailings and naphthenic acids from commercial mixtures.

These differences were observed in the relative distributions of naphthenic acids with low and high molecular masses. Similarly, Clemente and Fedorak (2004) followed the biodegradation of Kodak salts and Merichem acids with enrichment cultures analyzing the *tert*-butyldimethylsilyl derivatives of naphthenic acids by GC-MS. Additionally, Clemente et al. (2003a) developed a statistical method using arcsine-transformed to compare the distributions of naphthenic acids from various sources based on carbon numbers and *Z* families in the three-dimensional graphs.

Table 1.1 Expected nominal $[M+57]^+$ masses of *tert*-butyldimethylsilyl derivatives of naphthenic acids that fulfill the given formula $C_nH_{2n+Z}O_2$, based on carbon number (5 to 33) and *Z* families (0 to -12). Adapted from Clemente and Fedorak (2004).

Carbon number *n*	***Z* Number**						
	0	**-2**	**-4**	**-6**	**-8**	**-10**	**-12**
5	159 $C_5H_{10}O_2$						
6	173 $C_6H_{12}O_2$						
7	187 $C_7H_{14}O_2$	185 $C_7H_{12}O_2$					
8	201 $C_8H_{16}O_2$	199 $C_8H_{14}O_2$					
9	215 $C_9H_{18}O_2$	213 $C_9H1_6O_2$					
10	229 $C_{10}H_{20}O_2$	227 $C_{10}H_{18}O_2$	225 $C_{10}H_{16}O_2$				
11	243 $C_{11}H_{22}O_2$	241 $C_{11}H_{20}O_2$	239 $C_{11}H_{18}O_2$				
12	257 $C_{12}H_{24}O_2$	255 $C_{12}H_{22}O_2$	253 $C_{12}H_{20}O_2$	251 $C_{12}H_{18}O_2$			
13	271 $C_{13}H_{26}O_2$	269 $C_{13}H_{24}O_2$	267 $C_{13}H_{22}O_2$	265 $C_{13}H_{20}O_2$			
14	285 $C_{14}H_{28}O_2$	283 $C_{14}H_{26}O_2$	281 $C_{14}H_{24}O_2$	279 $C_{14}H_{22}O_2$	277 $C_{14}H_{20}O_2$		
15	299 $C_{15}H_{30}O_2$	297 $C_{15}H_{28}O_2$	295 $C_{15}H_{26}O_2$	293 $C_{15}H_{24}O_2$	291 $C_{15}H_{22}O_2$		
16	313 $C_{16}H_{32}O_2$	311 $C_{16}H_{30}O_2$	309 $C_{16}H_{28}O_2$	307 $C_{16}H_{26}O_2$	305 $C_{16}H_{24}O_2$	303 $C_{16}H_{22}O_2$	
17	327 $C_{17}H_{34}O_2$	325 $C_{17}H_{32}O_2$	323 $C_{17}H_{30}O_2$	321 $C_{17}H_{28}O_2$	319 $C_{17}H_{26}O_2$	317 $C_{17}H_{24}O_2$	
18	341 $C_{18}H_{36}O_2$	339 $C_{18}H_{34}O_2$	337 $C_{18}H_{32}O_2$	335 $C_{18}H_{30}O_2$	333 $C_{18}H_{28}O_2$	331 $C_{18}H_{26}O_2$	329 $C_{18}H_{24}O_2$
19	355 $C_{19}H_{38}O_2$	353 $C_{19}H_{36}O_2$	351 $C_{19}H_{34}O_2$	349 $C_{19}H_{32}O_2$	347 $C_{19}H_{30}O_2$	345 $C_{19}H_{28}O_2$	343 $C_{19}H_{26}O_2$
20	369 $C_{20}H_{40}O_2$	367 $C_{20}H_{38}O_2$	365 $C_{20}H_{36}O_2$	363 $C_{20}H_{34}O_2$	361 $C_{20}H_{32}O_2$	359 $C_{20}H_{30}O_2$	357 $C_{20}H_{28}O_2$
21	383 $C_{21}H_{42}O_2$	381 $C_{21}H_{40}O_2$	379 $C_{21}H_{38}O_2$	377 $C_{21}H_{36}O_2$	375 $C_{21}H_{34}O_2$	373 $C_{21}H_{32}O_2$	371 $C_{21}H_{30}O_2$
22	397 $C_{22}H_{44}O_2$	395 $C_{22}H_{42}O_2$	393 $C_{22}H_{40}O_2$	391 $C_{22}H_{38}O_2$	389 $C_{22}H_{36}O_2$	387 $C_{22}H_{34}O_2$	385 $C_{22}H_{32}O_2$
23	411 $C_{23}H_{46}O_2$	409 $C_{23}H_{44}O_2$	407 $C_{23}H_{42}O_2$	405 $C_{23}H_{40}O_2$	403 $C_{23}H_{38}O_2$	401 $C_{23}H_{36}O_2$	399 $C_{23}H_{34}O_2$
24	425 $C_{24}H_{48}O_2$	423 $C_{24}H_{46}O_2$	421 $C_{24}H_{44}O_2$	419 $C_{24}H_{42}O_2$	417 $C_{24}H_{40}O_2$	415 $C_{24}H_{38}O_2$	413 $C_{24}H_{36}O_2$
25	439 $C_{25}H_{50}O_2$	437 $C_{25}H_{48}O_2$	435 $C_{25}H_{46}O_2$	433 $C_{25}H_{44}O_2$	431 $C_{25}H_{42}O_2$	429 $C_{25}H_{40}O_2$	427 $C_{25}H_{38}O_2$
26	453 $C_{26}H_{52}O_2$	451 $C_{26}H_{50}O_2$	449 $C_{26}H_{48}O_2$	447 $C_{26}H_{46}O_2$	445 $C_{26}H_{44}O_2$	443 $C_{26}H_{42}O_2$	441 $C_{26}H_{40}O_2$
27	467 $C_{27}H_{54}O_2$	465 $C_{27}H_{52}O_2$	463 $C_{27}H_{50}O_2$	461 $C_{27}H_{48}O_2$	459 $C_{27}H_{46}O_2$	457 $C_{27}H_{44}O_2$	455 $C_{27}H_{42}O_2$
28	481 $C_{28}H_{56}O_2$	479 $C_{28}H_{54}O_2$	477 $C_{28}H_{52}O_2$	475 $C_{28}H_{50}O_2$	473 $C_{28}H_{48}O_2$	471 $C_{28}H_{46}O_2$	469 $C_{28}H_{44}O_2$
29	495 $C_{29}H_{58}O_2$	493 $C_{29}H_{56}O_2$	491 $C_{29}H_{54}O_2$	489 $C_{29}H_{52}O_2$	487 $C_{29}H_{50}O_2$	485 $C_{29}H_{48}O_2$	483 $C_{29}H_{46}O_2$
30	509 $C_{30}H_{60}O_2$	507 $C_{30}H_{58}O_2$	505 $C_{30}H_{56}O_2$	503 $C_{30}H_{54}O_2$	501 $C_{30}H_{52}O_2$	499 $C_{30}H_{50}O_2$	497 $C_{30}H_{48}O_2$
31	523 $C_{31}H_{62}O_2$	521 $C_{31}H_{60}O_2$	519 $C_{31}H_{58}O_2$	517 $C_{31}H_{56}O_2$	515 $C_{31}H_{54}O_2$	513 $C_{31}H_{52}O_2$	511 $C_{31}H_{50}O_2$
32	537 $C_{32}H_{64}O_2$	535 $C_{32}H_{62}O_2$	533 $C_{32}H_{60}O_2$	531 $C_{32}H_{58}O_2$	529 $C_{32}H_{56}O_2$	527 $C_{32}H_{54}O_2$	525 $C_{32}H_{52}O_2$
33	551 $C_{33}H_{66}O_2$	549 $C_{33}H_{64}O_2$	547 $C_{33}H_{62}O_2$	545 $C_{33}H_{60}O_2$	543 $C_{33}H_{58}O_2$	541 $C_{33}H_{56}O_2$	539 $C_{33}H_{54}O_2$

Recently, Vaz de Campos et al. (2006) characterized naphthenic acids from Brazilian heavy oil using the same approach as St. John et al. (1998). Their results showed the presence of alicyclic carboxylic acids and naphthenic acids with up to 4 rings in the molecule.

In addition to the methods base upon the work of St. John et al. (1998), direct characterization of naphthenic acids by negative ion atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) coupled to MS, MS/MS or FTICR MS has been reported. Hsu et al. (2000) measured the molecular distribution of naphthenic acids from commercial mixtures (Fluka, Kodak, Pfaltz and Bauer, and TCI America) and from a Californian crude oil with high acid content (4.06 mg KOH g^{-1}) using soft ionization techniques without derivatization. These techniques were evaluated in positive ion and negative ion modes and included chemical ionization (CI), liquid secondary-ion mass spectrometry (LSI-MS), APCI, and ESI. Among these techniques, negative ion APCI produced the cleanest spectra with high sensitivity and high selectivity for naphthenic acids. These high sensitivity and high selectivity technique were demonstrated when the resulting mass spectra from the isolated acid fraction were similar to the mass spectra of the whole crude oil, indicating that negative ion APCI selectively ionized naphthenic acids.

Qian et al. (2001) were able to resolve the identity of more than 3000 crude acids containing O_2, O_3, O_4, O_2S, O_3S and O_4S from South American heavy petroleum by negative ion ESI high field FTICR mass spectrometry. They extended the approach to characterize naphthenic acids and a large range of compounds were detected in the crude acids with carbon numbers ranging from 15 to 55, cyclic structures with 1 to 6 rings and aromatics with 1 to 3 rings.

Headley et al. (2002) also used the ESI-MS method to determine the mass profile of commercial (Fluka) and oil sand naphthenic acids. Their method was applied to the detection and quantification of naphthenic acids in natural waters with a detection limit of 0.01 mg L^{-1}. Furthermore, Rudzinski and co-workers (2002) evaluated the capacity of ESI and APCI ionization techniques in negative ion mode to separate Maya crude oil into various classes and MS or MS/MS analyzed the fractions. Experimental results showed that ESI-MS gave greater sensitivity to determine ring type and carbon distribution over negative ion APCI. These results were in disagreement as the previously reported by Hsu and co-workers (2000), who reported higher sensitivity in negative ion APCI. This difference was mainly due to ammonia addition to the solvent mixture, which enhances Electrospray ionization. Negative ion ESI-MS/MS revealed the presence of alkylsulfonic species with small amounts of naphthenic acids. These sulfur-containing compounds were also found in Venezuelan (Qian et al. 2001) and California (Tomczyk et al. 2001) crudes.

Lo et al. (2003) determined the concentrations of naphthenic acids in aqueous samples by negative ion ESI-MS. A semi-quantitative analysis of naphthenic acids was accomplished by spiking the samples with different concentrations of cyclohexanebutyric acid. Also, Lo et al. (2006) evaluated the analysis of naphthenic acids by negative-ion ESI-MS and APCI-MS using six model naphthenic acids, two commercial mixtures (Fluka and Merichem) and authentic naphthenic acids extracted from tailings ponds water. Naphthenic acids samples were prepared in a mixture (50:50) of 2-propanol:water and were detected as protonated molecular ions under the APCI conditions. APCI gave the greater range of detection that can be useful for fingerprinting of naphthenic acids, whereas ESI gave the greater detection limit with the lowest operation cost.

Lately, new instrumental techniques have been used to characterize the complex mixture of naphthenic acids. Gabryelski and Froese (2003) analyzed naphthenic acids from various sources using negative ion ESI high-field asymmetric waveform ion mobility spectrometry (FAIMS). This powerful technique used FAIMS combined with quadrupole and tandem mass spectrometry time of flight (TOF) to characterize naphthenic acids from commercial and oil sands mixtures dissolved in methanol. FAIMS separates the ions in the gas phase prior to their introduction into the mass spectrometer. This separation is based on their mobility at high and low electric field and is sensitive to the ion structure. Separated ions were detected by quadrupole MS and tandem mass spectrometry (TOF). Quadrupole MS proved to be useful for the characterization of fragile ions that cannot be detected by any other methods and TOF-MS/MS provided insight into the naphthenic acids structures.

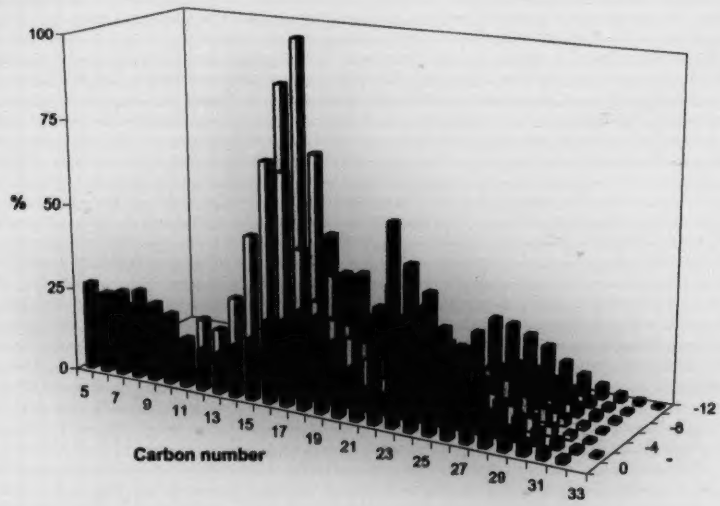
Using negative ion nanospray Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, Barrow et al. (2003) provided insight into the acidic species present in two West African crude oil samples. Mass spectra of both samples showed similar *m/z* values with different intensities and the presence of two signals per integer *m/z*. These doublets were easily resolved and attributed to naphthenic acids with high hydrogen deficiency. They concluded that FTICR-MS was a well-suited method to characterize naphthenic acids within the crude oil samples because it allowed the selective observation of deprotonated naphthenic acids due to the high mass accuracy and high resolution of FTICR mass spectrometry. Later, Barrow et al. (2004) used the same approach to characterize two commercial and one oil sand naphthenic acid samples by negative ion FTICR-MS. Their results were presented in plots that showed the relative intensities of the different naphthenic acids classes detected as a function of carbon content. These plots gave an insight into the naphthenic acids present in the samples and can be used as fingerprints to trace the source of naphthenic acids. In addition, these plots showed the complexity of the oil sands naphthenic acids that predominantly yielded compounds with Z =-4, -6 and -12.

Hao et al. (2005) applied a two-dimensional gas chromatography time of flight mass spectrometry (GC x GC/TOF-MS) to characterize naphthenic acids in two commercial mixtures (Fluka and Acros) and one naphthenic acids mixture extracted from Syncrude tailings. They generated two-dimensional contour plots with a gradient of color that represented the intensities of the corresponding methylated ions using mass deconvolution software. The contour plots provided specific patterns for acyclic and monocyclic naphthenic acids that can be used to determine the source of naphthenic acids. Characteristics patterns were observed in Fluka and Acros naphthenic acids plots with abundance of low molecular weight compounds, whereas Syncrude naphthenic acids plots showed fewer Z=0 and -2 homologues. In addition, co-elution of naphthenic acids with more than one ring showed characteristic bright zones that decreased in intensity with the number of rings until little signal was perceived for Z values less than -8. These findings were in disagreement with previous studies (Barrow et al. 2004) that reported naphthenic acids with Z=-12 for an oil sand sample.

A sensitive and specific HPLC method with QTOF-MS detection was developed to characterize the complex mixture of naphthenic acids (Bataineh et al. 2006). This method combines the high selectivity of the HPLC to separate naphthenic acids isomers based on carbon number and *Z* series with the high resolution of the QTOF-MS that enables the detection of oxidized products in the same analysis. Similar characterization profiles were acquired with refined Merichem naphthenic acids by QTOF as was previously displayed by GC low resolution mass spectrometry (LRMS); in both procedures, no naphthenic acids were detected with $n > 20$. However, more significant differences were observed when comparing profiles of naphthenic acids extracted from tailings ponds. Although the GC-LRMS profile showed naphthenic acids with low and high molecular weight (Figure 1.7A), the high molecular weight acids were absent in the HPLC-QTOF-MS profile (Figure 1.7B). These differences were the result of "double derivatization" that occurs when a naphthenic acid contains an additional reactive H (such as in a hydroxyl group). This reactive H and the carboxylic H both react with the derivatizing agent, forming a high-molecular weight "double derivative". Upon fragmentation in the LRMS, the major fragments are misassigned as high molecular weight compounds that were classify by Holowenko et al. (2001) as "C22+ cluster". However, in the newly developed HPLC-QTOF-MS method of Bataineh et al. (2006) as derivatization is not required, naphthenic acids with $n > 20$ were not identified.

Bataineh et al. (2006) also reported the presence of naphthenic acids with three oxygens that were clearly detected by QTOF and confirmed by GC high-resolution mass spectrometry (HRMS).

A) GC-LRMS



B) HPLC/QTOF-MS

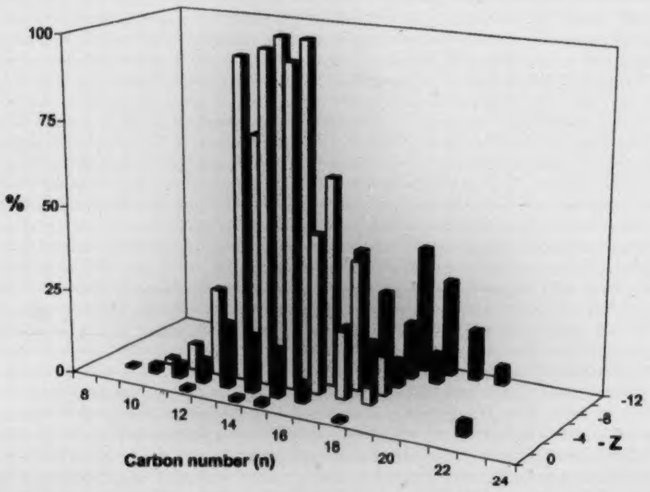


Figure 1.7 Example of the three-dimensional graphs of extracted naphthenic acids from Syncrude tailings water analyzed by GC-LRMS (A) and by HPLC/QTOF-MS (B), showing the relative intensity versus carbon number and *Z* families taken from Bataineh et al. (2006).

Furthermore, in biodegradation studies oil sands naphthenic acids were less biodegradable than commercial naphthenic acids, as reported by Scott et al. (2005). Mass spectra of partially degraded oil sands acids naphthenic acids showed the presence of highly alkyl-substituted compounds, which were recalcitrant to biodegradation. Clearly, there has been an increasing interest to determine the molecular composition of naphthenic acids in order to understand corrosion in refineries, toxicity and fate in the environment. Even with the most sophisticated MS techniques individual naphthenic acids had not been identified. However, operating in the negative ion mode gives better results than the positive ion mode and it seems that FTICR and ESI-FAIMS are the most promising methods to elucidate naphthenic acids structures.

1.3 Process-affected Waters by Oil Sands Operations

The separation of bitumen from the oil sands by hot water extraction produces large volumes of fine tailings that are hydraulically transported to large settling ponds where they slowly densify and dewater producing process-affected water that is recycled back into the extraction process (MacKinnon et al. 1989). It is an estimate that about 70% of the Syncrude water demand is supplied with recycled water (MacKinnon and Boerger 1986). However, recycling of the water yields elevated concentrations of dissolved ions and naphthenic acids that give the tailings waters their unique characteristics. Elevated concentrations of sodium, sulfates and chlorine are found in natural waters impacted by tailings waters (MacKinnon et al. 2004).

Some researchers have used the presence of naphthenic acids and major ions such as Na^+ and Cl^- as indicators of water that has been in contact with oil sands. For example, MacKinnon and co-workers (2004) reported some migration of process-affected waters from the East Toe Bern (ETB), a tailing deposit constructed on the eastern dyke of the Mildred Lake Settling Basin (MLSB), into surface waters affecting the Beaver Creek Valley and possible groundwater systems. Similarly, Gervais (2004) monitored three segments of shallow quaternary sand/gravel aquifers around the mining site potentially impacted by process-affected water. Gervais (2004) reported that process-affected waters mostly impacted two of those aquifers. Oiffer (2004) studied several groundwater plumes at Syncrude Canada Ltd. site. In that study, 14 groundwater samples were collected around the MLSB site and were characterized according to their impact by process-affected waters. Almost all the samples in the study by Oiffer (2004) were classified as impacted by process water.

The next two sub-sections will discussed the possible mechanism of introduction of naphthenic acids to surface waters and groundwaters, concentrations of naphthenic acids and the main water reservoirs at the Athabasca oil sands.

1.3.1 Surface Waters

Surface waters in the oil sands mining area may acquire naphthenic acids through different process including, effluent discharge, natural leaching of exposed oil sands deposits, seepage from holding ponds, industrial spills or crude oil refinery release (Headley and McMartin 2004), however, the most significant concentrations of naphthenic acids are found in waters that have direct contact with the oil sands material and the concentrations of naphthenic acids found in these effluents may reach 120 mg L^{-1} (Holowenko et al. 2001).

The Athabasca River and its tributaries are the main natural surface waters that cut through the oil sands deposits. This river is naturally exposed to naphthenic acids by runoff water from precipitation or as a result of leaching from the ores (Leung et al. 2003). However, the concentrations of naphthenic acids found in various locations along the Athabasca River are at background levels ranging from 0.1 to 0.9 mg L^{-1} (Schramm et al. 2000). Syncrude Canada Ltd. pumps water from the Athabasca River into the Mildred Lake, which is a natural lake adjacent to the active mine areas modified to serves as a clean water reservoir for Syncrude's extractions. Concentrations of naphthenic acids in Mildred Lake detected by FTIR are in the background level < 2 mg L^{-1} (Leung et al. 2003).

Over the many years of operation under a zero discharge policy, the oil sands surface mining has produced large volumes of tailings. For example, in 1998 Syncrude Canada Ltd. alone produced 75 million m^3 of tailings (Li and Fung 1998) and it is estimate that about 10^9 m^3 of oil sands process-affected waters are being held on the mining area and must be reclaimed (Del Rio et al. 2006). Consequently, Suncor Energy Inc. and Syncrude Canada Ltd. have constructed various experimental ponds and wetlands to study the sustainability of the "wet landscape" to produce environmentally accepted water (Gentes et al. 2006).

Hadwin et al. (2006) classified 12 wetlands located in a radius of 50 km of Fort McMurray according to their input of process-affected waters and concentrations of naphthenic acids in the free water zone. Based on the results, Hadwin et al. (2006) classified the wetlands in three groups: high-impacted wetlands with concentrations of naphthenic acids that range from 45 to 80 mg L^{-1}, low-impacted wetlands with concentrations between 4 to 12 mg L^{-1} and non-impacted wetlands with average concentrations of naphthenic acids ≤ 2 mg L^{-1}.

Alternatively, the mine companies have created shallow pits to store tailings waters for 1 or more years under aerobic conditions to improve the water quality by natural processes (MacKinnon and Boerger 1986). Examples of naturally aged ponds are MLSB7, which was aged for seven years and Pit #5, which was aged eleven years without input from process-affected waters, and both ponds showed a reduction in toxicity (Holowenko et al. 2001).

In addition, Syncrude Canada Ltd. has established a seepage control system to ensure that process-affected waters resulting from the bitumen extraction are kept on their sites rather than be released into the surface waters. This seepage control is composed of berms and ditches that collect dyke seepage and runoff water from the Mildred Lake Settling Basin (MLSB), the main active settling basin at Syncrude's lease that receives input from the bitumen extraction and upgrading processes. Using engineering systems, the water from the seepage control is returned back to MLSB (MacKinnon et al. 2004).

At present, most of the large volumes of surface waters affected by the mining operations are held in settling ponds around the mining area and the rest is distributed among wetlands, ponds, pits and ditches. These surface waters often have different physical and chemical characteristics that depend on the input of waters that they received, time of storage, and depth of the pond. Characteristics of some of these waters and storage ponds have been described in other studies (van den Heuvel et al. 1999a, 1999b, Leung et al. 2003 and Del Rio et al. 2006).

1.3.2 Groundwater

Groundwater aquifers have also been impacted with naphthenic acids from process-affected waters. Typical concentrations of naphthenic acids found in groundwater samples close to the oil sands tailings ponds range from 0.4 to 51 mg L^{-1} (MacKinnon et al. 2004) and in groundwater samples from basal and limestone aquifers concentrations may exceed 55 mg L^{-1} (Headley et al. 2002). The detailed process by which these acids are introduced into groundwater systems is not well understood. However, some researchers suggested that the main processes by which groundwater aquifers become impacted with naphthenic acids are: leaching from the oil sands, infiltration from tailings basins or seepage from dyked tailings (Gervais 2004).

For instance, Gervais (2004) monitored the attenuation of naphthenic acids during the groundwater flow in southwest aquifer at Suncor Pond 2/3 site, MLSB at Syncrude site and the Muskeg River mine at the Albian site. Based on the concentrations of naphthenic acids and the ratio Na^+: Cl^-, the groundwater samples were classified as impacted by process waters, possibly impacted or non-impacted using the tree and index classification. The tree scheme is showing in Figure 1.8 as modified by Oiffer (2004) and the index method assigns numeric values to each groundwater sample according to the concentration of naphthenic acids, molar ratio (Na^+: Cl^-) and sodium concentration. In this classification < 30% indicates non-contaminated samples, 30 to 75% means possible process-affected and > 75% means definitely process-affected. Most of the groundwater samples collected from Suncor Pond 2/3 and MLSB resulted impacted by process waters and showed concentrations from 0.4 to 51 mg L^{-1}, whereas groundwater samples collected from the Muskeg River mine site showed background concentrations of naphthenic acids as determine by FTIR.

Similarly, Oiffer (2004) characterized the water of 14 groundwater samples collected around the MLSB active pond and ETB at Syncrude's site. Oiffer (2004) used the same classification as Gervais (2004), and based on that classification, seven groundwater samples were impacted by process-affected waters with concentrations of naphthenic acids from 30 to 80 mg L-1, three groundwater samples were possibly impacted by process-affected with concentrations of naphthenic acids from 2 to 12 mg L-1 and two represent background conditions with concentrations of naphthenic acids < 0.5 mg L-1 indicating that these samples were affected by natural process rather than by mining operations. Clearly, these studies indicate that the input of naphthenic acid in groundwater systems is the result of both natural and mining process.

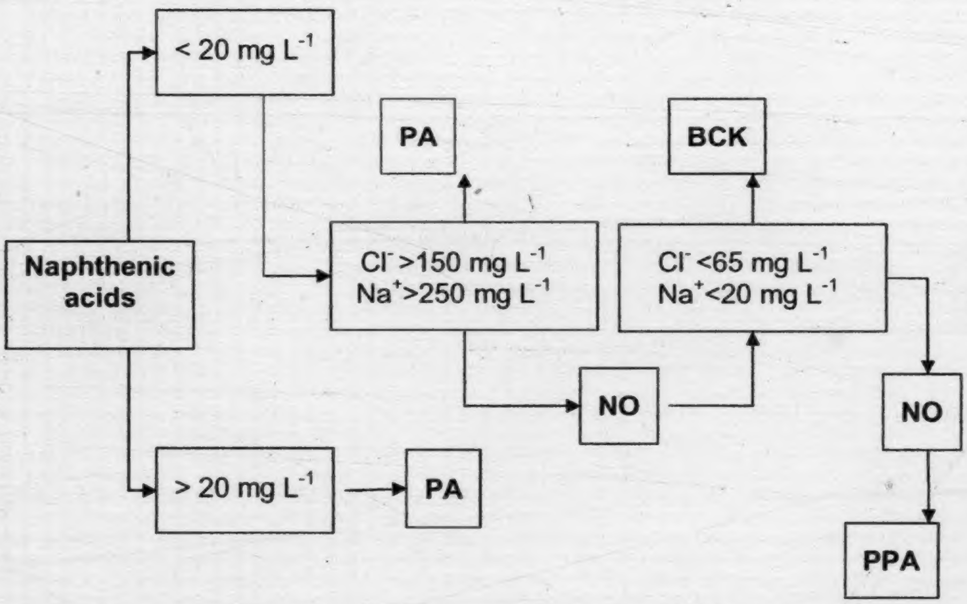


Figure 1.8 Tree classification scheme to identify groundwater containing process-affected water from tailings pond, where BCK= background concentrations, PPA = possibly process-affected and PA= process-affected. Adapted from Oiffer (2004).

1.4 Objectives

Previous work with naphthenic acids has concentrated on the development of analytical methods to characterize naphthenic acids mixtures or to measure their concentrations in process-affected waters and in biodegradation studies. Unfortunately, the current methods to detect naphthenic acids are not specific because they respond to all naturally occurring carboxylic acids. In addition, naphthenic acids are toxic to animals and plants, and there have been no reports about the specific detection of these acids in animals or plants exposed to naphthenic acids because of the lack of methodology to extract naphthenic acids from tissues. Therefore, there is a need to develop more specific and sensitive analytical methods to specifically detect naphthenic acids in waters and to develop methods for extraction of naphthenic acids from animal serum, tissues and plants.

At the beginning of this study, it was hypothesized that some isomers of naphthenic acids with values of *Z*=-4(two rings) or *Z*=-6 (three rings) would be key in the detection of these acids by GC-MS, because they are abundant in naphthenic acids mixtures and fatty acids with *Z*=-4 or *Z*=-6 are rare to be found in nature as complex mixtures. St. John et al. (1998) developed the GC-MS method used in this study to characterize naphthenic acids mixtures from commercial sources based on their *tert*-butyldimethylsilyl esters of naphthenic acids. Subsequently, Holowenko et al. (2002) applied this method to analyze naphthenic acids extracted from oil sands process-affected waters and later Clemente (2004), used the same approach to characterize oil sands ores and in biodegradation studies.

The specific objectives of this study were therefore to:

1. Develop a method for extraction of naphthenic acids from water samples, rat tissues, plasma, and plant tissues.

2. Apply the GC-MS method developed by St. John et al. (1998) to specifically detect naphthenic acids in water, rat tissues, plasma and plant tissues extracts.

1.5 Thesis Overview

This thesis is organized in five chapters. Chapter 1 contains general information about the bitumen extraction at the Athabasca oil sands, naphthenic acids description and literature review of the recent methods available for the analyses of naphthenic acids. Chapter 2 describes a study to detect naphthenic acids in water samples. Providing the method for the extraction and analysis of naphthenic acids by GC-LRMS and GC-HRMS. Chapter 3 contains the preliminary work of the detection of naphthenic acids in plant tissue. Chapter 4 describes a study to detect naphthenic acids in rat tissues and plasma work, the method for extraction of naphthenic acids in both matrices and the application of the GC-LRMS and GC-HRMS method to detect naphthenic acids (as outlined in Chapter 2) in rats fed with Merichem naphthenic acids. Chapter 5 is a summary of the method developed to detect naphthenic acids in different matrices, providing concluding remarks and future directions.

1.6 Literature Cited

AME. 2005. Alberta Ministry of Energy 2004-2005 Annual Report [online]. Available from http://www.energy.gov.ab.ca/docs/aboutus/pdfs/AR2005.pdf [cited December 5 2006].

Barnes, H.M., Amburgey, T.L. and Sanders, M.G. 2005. Performance of copper naphthenate and its analogs as ground contact wood preservatives. Bioresource Technology, 96: 1131-1135.

Barrow, M.P., McDonnell, L.A., Feng, X., Walker, J. and Derrick, P.J. 2003. Determination of the nature of naphthenic acids present in crude oils using nanospray Fourier transform ion cyclotron resonance mass spectrometry: the continued battle against corrosion. Analytical Chemistry, 75: 860-866.

Barrow, M.P., Headley, J.V., Peru, K.M. and Derrick, P.J. 2004. Fourier transform ion cyclotron resonance mass spectrometry of principal components in oil sands naphthenic acids. Journal of Chromatography A, 1058: 51-59.

Bataineh, M., Scott, A.C., Fedorak, P.M. and Martin, J.M. 2006. Capillary HPLC/QTOF-MS for characterizing complex naphthenic acid mixtures and their microbial transformation. Analytical Chemistry, 78: 8354-8361.

Bendell-Young, L.I., Bennnett, K.E., Crowe, A., Kennedy, C.J., Kermode, A.R., Moore, M.M., Plant, A.L. and Wood, A. 2000. Ecological characteristics of wetlands receiving and industrial effluent. Ecological Applications, 10: 310-322.

Brient, J.A., Wessner, P.J. and Doly, M.N. 1995. Naphthenic acids. In: Kroschwitz, J.I. (Ed.). Encyclopedia of Chemical Technology, 4th ed. Vol. 16. John Wiley and Sons, New York, NY, pp. 1017-1029.

Chastko, P. 2004. Developing Alberta's Oil Sands from Karl Clark to Kyoto. Published by the University of Calgary Press. Calgary, Canada.

Clark, K.A. and Pasternack, D.S. 1932. Hot water separation of bitumen from Alberta bituminous sand. Industrial and Engineering Chemistry, 24: 1410-1416.

Clemente, J.S. 2004. The characterization analyses and biodegradation of naphthenic acids. MSc. Thesis, University of Alberta, Edmonton, Canada.

Clemente, J.S. and Fedorak, P.M. 2004. Evaluation of the analyses of *tert*-butyldimethylsilyl derivatives of naphthenic acids by gas chromatography-electron impact mass spectrometry. Journal of Chromatography A, 1047: 117-128.

Clemente, J.S. and Fedorak, P.M. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere, 60: 585-600.

Clemente, J.S., Prasad, N.G.N., MacKinnon, M.D. and Fedorak, P.M. 2003a. A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. Chemosphere, 50: 1265-1274.

Clemente, J.S., Yen, T.-Y. and Fedorak, P.M. 2003b. Development of a high performance liquid chromatography method to monitor the biodegradation of naphthenic acids. Journal of Environmental Engineering and Science, 2: 177-186.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology, 38: 1009-1016.

CONRAD. 1998. Naphthenic acids background information discussion report. Alberta Oil Sands Technology and Research Authority, Calgary, AB. June 1998. 65 pp+ Appendices.

Crowe, A.U., Han, B., Kermode, A.R., Bendell-Young, L.I. and Plant, A.L. 2001. Effects of oil sands effluent on cattail and clover: photosynthesis and the level of stress proteins. Environmental Pollution, 113: 311-322.

Del Rio, L.F., Hadwin, A.K.M., Pinto, L.J., MacKinnon, M.D. and Moore, M.M. 2006. Degradation of naphthenic acids by sediment micro-organisms. Journal of Applied Microbiology, 101: 1049 -1061.

Dokholyan, B.K. and Magomedov, A.K. 1983. Effect of sodium naphthenate on survival and some physiological-biochemical parameters of some fishes. Journal of Ichthyology, 23: 125-132.

du Preez, A.C. and Preston, J.S. 1992. The solvent extraction of rare-earth metals by carboxylic acids. Solvent Extraction and Ion Exchange, 10: 207-230.

Dzidic, I., Somerville, A.C., Raia, J.C. and Hart, H.V. 1988. Determination of naphthenic acids in California crudes and refinery wastewaters by fluoride-ion chemical ionization mass-spectrometry. Analytical Chemistry, 60: 1318-1323.

Fan, T.P. 1991. Characterization of naphthenic acids in petroleum by fast-atom-bombardment mass-spectrometry. Energy and Fuels, 5: 371-375.

Frank, R.A., Kavanagh, R., Burnison, B.K., Headley, J.V., Peru, K.M., Van Der Kraak, K. and Solomon, K.R. 2006. Diethylaminoethyl-cellulose clean-up of a large volume naphthenic acid extract. Chemosphere, 6: 1346-1352.

Gabryelski, W. and Froese, K.L. 2003. Characterization of naphthenic acids by electrospray ionization high-field asymmetric waveform ion mobility spectrometry mass spectrometry. Analytical Chemistry, 75: 4612-4623.

Gentes, M.L, Waldner, C., Papp, Z. and Smits, J.E.G. 2006. Effects of oil sands tailings compounds and harsh weather on mortality rates, growth and detoxification efforts in nestling tree swallows (*Tachycineta bicolor*). Environmental Pollution, 142: 24-33

Gervais, F.J.M. 2004. Fate and transport of naphthenic acids in a glacial aquifer. MSc. Thesis, University of Waterloo, Waterloo, Canada.

Green, J.B., Yu, S.K.-T. and Vrana, R.P. 1994. GC-MS analysis of carboxylic acids in petroleum after esterification with fluoroalcohols. Journal of High Resolution Chromatography, 17: 427-437.

Hadwin, A.K.M., Del Rio, L.F., Pinto, L.J., Painter, M., Routhlege, R. and Moore, M.M. 2006. Microbial communities in wetlands of the Athabasca oil sands: genetic and metabolic characterization. FEMS Microbiology Ecology, 55: 68-78.

Hao, C., Headley, J., Peru, K.M., Frank, R., Yang, P. and Solomon, K.R. 2005. Characterization and pattern recognition of oil-sand naphthenic acids using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. Journal of Chromatography A, 1067: 277-284.

Headley, J.V. and McMartin, D.W. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. Environmental Science and Health A: 1989-2010.

Headley, J.V., Peru, K.M., McMartin, D.W. and Winkler, M. 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. Journal of AOAC International, 85: 182-187.

Herman, D.C., Fedorak, P.M., MacKinnon, M.D. and Costerton, J.W. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Canadian Journal of Microbiology, 40: 460-477.

Holowenko, F.M. and Fedorak, P.M. 2001. Evaluation of gas chromatography - electron impact mass spectrometry method for characterizing naphthenic acids. University of Alberta, Edmonton.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2001. Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research, 35: 2595-2606.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research, 36: 2843-2855.

Hsu, C.S., Dechert, G.J., Robbins, W.K. and Fukuda, E.K. 2000. Naphthenic acids in crude oils characterized by mass spectrometry. Energy and Fuels, 14: 217-223.

Jivraj, M.N., MacKinnon, M. and Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Analytical Methods Manual. 4th ed. Research Department, Syncrude Canada Ltd., Edmonton, Canada.

Kamaluddin, M. and Zwiazek, J.J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. Tree Physiology, 22: 1265-1270.

Kasperski, K.L. 1992. A review of properties and treatment of oil sands tailings. AOSTRA Journal of Research, 8: 11-52.

Li, X. and Fung, M.Y.P. 1998. Creating soil-like materials for plant growth using tailings sand and fine tails. Journal of Canadian Petroleum Technology, 37: 44-47.

Leung, S.S., MacKinnon, M.D. and Smith, R.E.H. 2003. The ecological effects of naphthenic acids and salts on phytoplankton from the Athabasca oil sands region. Aquatic Toxicology, 62: 11-26.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. 2003. Electrospray-mass spectrometric analysis of reference carboxylic acids and Athabasca oil sands naphthenic acids. Analytical Chemistry, 75: 6394-6400.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. 2006. Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. Water Research, 40: 655-634.

MacKinnon, M.D. 1989. Development of the tailings pond at Syncrude's oil sands plant: 1978-1987. AOSTRA Journal of Research, 5: 109-133.

MacKinnon, M.D. and Boerger, H. 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Canadian Journal of Water Pollution and Research, 21: 496-512.

MacKinnon, M., Kampala, G., Marsh, B., Fedorak, P. and Guigard, S. 2004. Indicators for assessing transport of oil sands process-affected waters. Proceedings of the 4th International Groundwater Quality Conference, University of Waterloo, Waterloo, Canada. July 2004. IAHS publication 297. pp. 71-80.

Masliyah, J. 2002. Processing of tailings in Canadian oil sands industry. Transaction of Nonferrous Metals Society of China, 12: 524-528.

Mustafaev, S.A. and Shikhalizade, P.D. 1980. Physicochemical properties of esters of high-molecular-weight naphthenic acids. Chemistry and Technology of Fuels and Oils, 16: 71-74.

Nascimento, L.R., Reboucas, L.M.C., Koike, L., Reis, F.D.M., Soldan, A.L., Cerqueira, J.R. and Marsaioli, A.J. 1999. Acidic biomarkers from Albacora Oils, Campos Basin, Brazil. Organic Geochemistry, 30: 1175-1191.

Oiffer, A.A.L. 2004. The fate and transport of toxic and potentially harmful components of process-affected groundwater - Fall 2004 field investigation. University of Waterloo, Waterloo, Canada.

Peng, J., Headley, J.V. and Barbour, S.L. 2002. Adsorption of single-ring model naphthenic acids on soils. Canadian Geotechnical Journal, 39: 1419-1426.

Peters, L.E. 1999. The effects of oil sands aquatic reclamation on the early-life stages of fish. MSc. Thesis, University of Waterloo, Waterloo, Canada

Qian, K., Robbins, W.K., Hughey, C.A., Cooper, H.J., Rodgers, R.P. and Marshall, A.G. 2001. Resolution and identification of elemental compositions for more than 3000 crude acids in heavy petroleum by negative-ion microelectrospray mass spectrometry. Energy and Fuels, 15: 1505-1511.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J. and MacKinnon, M.D. 1999. Responses of boreal plants to high salinity oil sands tailings water. Journal of Environmental Quality, 28: 1957-1962.

Rogers, V.V., Wickstrom, M., Liber, K. and MacKinnon, M.D. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands. Toxicological Sciences, 66: 347-355.

Rogers, V.V., Liber, K. and MacKinnon, M.D. 2002b. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere, 48: 519-527.

Rudzinski, W.E., Oehlers, L. and Zhang, Y. 2002. Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy and Fuels, 16: 1178-1185.

Saab, J., Mokbel, I., Razzouk, A.C., Ainous, N., Zydowicz, N., and Jose, J. 2005. Quantitative extraction procedure of naphthenic acids contained in crude oils. Characterization with different spectroscopic methods. Energy and Fuels, 19: 545-531.

Seifert, W.K. and Teeter, R.M. 1969. Preparative thin-layer chromatography and high resolution mass spectrometry of crude oil carboxylic acids. Analytical Chemistry, 41: 786-795.

Schramm, L.L., Stasiuk, E.N. and MacKinnon, M.D. 2000. Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes. In: Surfactants: Fundamentals and Applications in the Petroleum Industry. Schramm, L.L. (Ed). Cambridge University Press. Cambridge, UK. pp. 365-430.

Scott, A.C., MacKinnon, M.D. and Fedorak, P.M. 2005. Naphthenic acids in Athabasca oil sands tailings water are less biodegradable than commercial naphthenic acids. Environmental Science and Technology, 39: 8388-8394.

Slavcheva, E., Shone, B. and Turnbull, A. 1999. Review of naphthenic acid corrosion in oil refining. British Corrosion Journal, 34: 125-131

St. John, W.P., Rughani, J., Green, S.A. and McGinnis, G.D. 1998. Analysis and characterization of naphthenic acids by gas chromatography electron impact mass spectrometry of *tert*-butyldimethylsilyl derivatives. Journal of Chromatography A, 807: 241-251.

Tissot, B.P. and Welte, D.H. 1978. Petroleum Formations and Occurrence. Springer-Verlag, New York, NY.

Tomczyk, N.A., Winans, R.E., Shinn, J.H. and Robinson, R.C. 2001. On the nature and origin of acidic species in petroleum. 1. Detailed acid type distribution in a California crude oil. Energy and Fuels, 15: 1498-1504.

van den Heuvel, M.R., Power, M., MacKinnon, M.D., Van Meer, T., Dobson, E.P. and Dixon, D. G. 1999a. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). I. Water quality characteristics and yellow perch physiological and population responses. Canadian Journal of Fisheries and Aquatic Science 56:1213-1225.

Vaz de Campos, M.C., ConceiÇão, E.O., Sanchez, P.J.F., Sartori, C.M.P. and Bastos, E.C. 2006. Analysis of *tert*-butyldimethylsilyl derivatives in heavy gas oil from Brazilian naphthenic acids by gas chromatography coupled to mass spectrometry with electron impact ionization. Journal of Chromatography A, 1105: 95-105.

Wong, D.C.L., van Compernolle, R., Nowlin, J.G., O'Neal, D.L. and Johnson, G.M. 1996. Use of superciritcal fluid extraction and fast ion bombardment mass spectrometry to identify toxic chemicals from a refinery effluent adsorbed onto granular activated carbon. Chemosphere, 32: 1669-1679.

Yang, X. and Czarnecki, J. 2005. Tracing sodium naphthenate in asphaltenes precipitated from Athabasca bitumen. Energy and Fuels, 19: 2455-2459.

Yen, T.-W., Marsh, W.P., MacKinnon, M.D. and Fedorak, P.M. 2004. Measuring naphthenic acids concentrations in aqueous environmental samples by liquid chromatography. Journal of Chromatography A, 1033: 83-90.

2. Detection of Naphthenic Acids in Water by Gas Chromatographic - Mass Spectrometry[*]

2.1 Introduction

Naphthenic acids comprise a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids, with the general chemical formula $C_nH_{2n+Z}O_2$, where *n* indicates the carbon number and *Z* is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation (Clemente and Fedorak 2005). The absolute value of *Z* divided by 2 gives the number of rings in the compounds. The rings may be fused or bridged. The acyclic components are highly branched, unlike fatty acids (Rudzinksi et al. 2002), although fatty acids fit the formula $C_nH_{2n+Z}O_2$, for $Z = 0$.

Naphthenic acids are natural components of petroleum (Seifert and Teeter 1969, Seifert et al. 1969, Fan 1991, Tomczyk et al. 2001) and Athabasca oil sands ore (Clemente 2004). Naphthenic acids in the Athabasca oil sands in Canada were produced by biodegradation of mature petroleum (Tissot and Welte 1978). The alkaline, hot water extraction process used by some of the oil sands companies, dissolves these acids as naphthenates which partition into the aqueous phase, and these become the major dissolved organic compounds in the tailings waters. Naphthenic acids have been shown to be toxic to a variety of organisms (see Clemente and Fedorak 2005 for a review), and these organic acids are the main contributors to the toxicity associated with the oil sands tailings waters.

---

[*] A version of this chapter has been published. Merlin, M., S.E. Guigard, and P.M. Fedorak. 2007. J. Chromatogr. A 1140: 225-229.

Because of the complexity of naphthenic acids mixtures, the separation, quantification, and identification of individual compounds has not been achieved. However, analytical methods have been developed to measure the total naphthenic acids concentrations in oil or water. These include the oil sands industry standard Fourier transform infrared (FTIR) spectroscopy method (Jivraj et al. 1995, Holowenko et al. 2001) and a recently developed high performance liquid chromatography (HPLC) method (Yen et al. 2004) which yields results that are similar to the FTIR method. The carboxylic acid portion of naphthenic acids is key to both of these quantification methods. In addition, various mass spectrometry methods have been developed to characterize the carboxylic acids in naphthenic acids by assigning ions with various masses to the general formula $C_nH_{2n+Z}O_2$, based on their carbon and Z numbers. These methods have been reviewed by Clemente and Fedorak (2005).

For over 30 years, two oil companies (Suncor Energy Inc., and Syncrude Canada Ltd.) have extracted bitumen from the oil sands ore. More recently, Albian Sands Energy Inc. has begun operations, and several other companies are planning new oil sands extraction plants. Water is an important component of the extract process and naphthenic acids are transferred from the ore to tailings waters. Because of the toxicity of naphthenic acids, the tailings waters cannot be released to receiving waters. Thus, vast tailings ponds have been formed to hold these waters. Seepage from these ponds can mix with surface waters creating so-called “process-affected” waters. There are a variety of ditches and additional storage ponds on each oil sands site to collect and hold process-affected waters.

Each of the oil sands extraction plants is adjacent to the Athabasca River or its tributaries, and there are concerns about possible seepage of the stored process-affected waters into these waterways. Because of the lack of specificity of the FTIR and HPLC methods, it is difficult to distinguish naphthenic acids in process waters from the naturally occurring organic acids in the natural waters. Thus, this study evaluates a gas chromatography-low resolution mass spectrometry (GC-LRMS) method to specifically detect naphthenic acids in natural waters.

The GC-LRMS method was developed by St. John et al. (1998) to characterize naphthenic acids from commercial and oil sands sources. Naphthenic acids were derivatized to yield their *tert*-butyldimethylsilyl esters, and electron impact mass spectrometry of the esters predominantly yields $[M+57]^+$ ions, where M is the mass of the underivatized naphthenic acids (St. John et al. 1998, Clemente and Fedorak 2004, Vaz de Campos et al. 2006). Holowenko et al. (2002) applied this method to analyze naphthenic acids extracted from oil sands process-affected waters, and this method has been used to study the naphthenic acids composition in various oil sands affected waters (Clemente et al. 2003), in oil sands ores (Clemente 2004) and in biodegradation studies (Clemente et al. 2004, Scott et al. 2005).

In this study, it was hypothesized that certain $[M+57]^+$ ions might be quite common and abundant in naphthenic acids from various sources. Three ions (m/z = 265, 267 and 279) that fit these criteria, and reconstructed (extracted) ion chromatograms (RICs, Murray et al. 2006) from GC-LRMS analyses could provide improved specificity to detect naphthenic acids. To test this method, a variety of water samples from various sources were extracted, derivatized and analyzed by GC-LRMS to determine if naphthenic acids could be detected in these waters. Selected samples were also brominated and analyzed by GC-high resolution MS (GC-HRMS) to further evaluate the results from GC-LRMS.

2.2 Materials and Methods

2.2.1 Naphthenic Acids Mixtures and Chemicals

The commercial preparation of naphthenic acids used in this study was Merichem acids obtained as a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX). Oil sand naphthenic acids were extracted from 2 L of water collected from the West-In Pit (WIP) tailings pond located at the Syncrude Canada Ltd. Mildred Lake site. WIP tailings water was stored at 4°C walk-in fridge and was extracted according to the method given by Holowenko et al. (2001). The extracted naphthenic acids were diluted in 50-mL of 0.1 M sodium hydroxide and the concentration of this alkaline solution was 930 mg L^{-1}, as determine by HPLC using the protocol developed by Yen et al. (2004). Suwannee River Fulvic acids reference materials (cat no. 1R101F) were purchased from the International Humic Substances Society (University of Minnesota, St. Paul, MN). Ore sample used in this study was provided by Dr MacKinnon from Syncrude lease.

2.2.2 Choosing Characteristic Ions for RICs

Over the years, approximately 60 samples of naphthenic acids from different sources including: commercial mixtures, oil sands ores and oil sands tailings waters from Syncrude, Suncor, and Albian Sands have been analyzed by GC-LRMS in different studies. Results from 34 randomly selected analyses of *tert*-butyldimethylsilyl esters of naphthenic acids were examined to find which $[M+57]^+$ ions were common and relatively abundant in these samples (Table 2.1). The designations and sources of the six commercially available naphthenic acids preparations (Group 1, Table 2.1) are as follows: "Merichem acids" were obtained as a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX), "Kodak acids" and "Kodak salts" were purchased from The Eastman Kodak Company (Rochester, NY), "Fluka acids" were obtained from Fluka Chemie (Buchs, Switzerland), and "Acros acids" were purchased from Acros Organics (Morris Plains, NJ).

The oil sands tailing waters (Group 2, Table 2.1) are from various oil sands companies, and the process-affected waters (Group 3, Table 2.1) originated from various seepage control ditches and ponds. Oil sands ores from several different locations are presented as Group 4 in Table 2.1.

2.2.3 Water Samples

For this study, 23 water samples were collected from three different provinces in Canada. These are summarized in Table 2.2, and they are grouped as waters from petroleum industry sources (samples 2 to 6), surface waters (samples 7 to 17), and groundwater (18 to 24). Approximately 4 L of each sample were collected in either Nalgene bottles or glass bottles and these were stored at 4°C until being extracted, derivatized, and analyzed by GC-LRMS. Color was measured by the method of Cuthbert and del Giorgio (1992).

Table 2.1 Percentages of ions corresponding to C13 Z =–4 (*m/z*=267), C13 Z=–6 (*m/z*=265) and C14 Z=–6 (*m/z*=279) in 34 different sources of naphthenic acids.

	Abundance (% of total ions in GC-MS analysis)			
Sources	C13 Z–4	C13 Z–6	C14 Z–6	Sum of abundances
Commercial preparations (Group 1)				
Merichem	8	2	2	12
Kodak Acids	13	2	1	16
Kodak Salts	4	1	2	7
Fluka	9	1	1	11
Acros	3	1	2	6
Oils sands process waters (Group 2)				
Syncrude Aurora (fresh)	2	2	3	7
Syncrude MLSB (fresh)	1	1	3	5
Syncrude WIP 1 (fresh)	2	2	4	8
Syncrude WIP 2	5	4	7	16
Syncrude sample 2	2	3	5	10
Syncrude process water:7 y old	2	2	3	7
Syncrude process water:11 y old	2	1	3	6
Syncrude process water:16 y old	1	1	3	5
Suncor Energy Inc.	6	4	8	18
Albian Sands Energy Inc.	2	2	4	8
Syncrude process-affected surface waters (Group 3)				
SCP-1	8	2	6	16
MLSB-OP	5	4	7	16
Dyke Seepage	10	2	4	16
SCD South	13	2	4	19
AO-Ditch-01	9	2	4	15
AO-0-OW04-07ETB	9	2	4	15
ETB#2	5	5	4	14
ETB-GD	4	1	4	9
Seepage Control Ditch North	15	2	4	21
Oil sands ores[a] (Group 4)				
Syncrude 1	2	1	5	8
Syncrude 2	1	2	2	5
Syncrude 3	2	3	2	7
Syncrude 4	16	1	2	19
Suncor 1	1	1	1	3
Suncor 2	2	1	1	4
True North 1	1	1	1	3
True North 2	1	1	1	3
Albian 1	2	2	3	7
Albian 2	0	1	2	3

[a] Results from Clemente (2004), SCP-1 Seepage Control Pond, ETB East Toe Bern, SDC Seepage Control Ditch.

2.2.4 Detection of Naphthenic Acids in Water that Contacted Oil Sands Ore

Various amounts of an oil sands ore sample (3.75 g, 750 mg and 125 mg) were contacted with individual 1.25-L portions of North Saskatchewan River (pH 8.5) water to determine if naphthenic acids could be detected in these water samples. The ore and river water mixtures were placed in an Erlenmeyer on a shaker table operating at 150 rpm. After 24 h of shaking, at room temperature (approximately 20°C), the water was filtered through Whatman no. 1 paper, and 1 L was extracted for naphthenic acids as outlined below.

2.2.5 Extraction of Naphthenic Acids from Water Samples

Free fatty acids and naphthenic acids were extracted from water samples using a modified method of Fatoki and Vernon (1989). In that study, chloroform gave the highest the extraction efficiency of 89%, so this solvent was used in the present study for the extraction of naphthenic acids. Briefly, 1 L of water was acidified to pH 2 with concentrated HCl, and then 150 g NaCl was dissolved into the sample. The water sample was then extracted with three 60-mL portions of chloroform (HPLC grade, Fisher, Fair Lawn NJ). The free carboxylic acids were separated from the lipid fraction by the extracting the chloroform phase with three 10-mL portions of an alkaline aqueous solution containing 4% (w v^{-1}) sodium carbonate (pH 11.6). The free carboxylic acids were recovered from the alkaline solution by acidifying with concentrated HCl to pH 2 and extracting three times with 10 mL of dichloromethane. The combined dichloromethane extracts were dried under nitrogen and the residue was dissolved in fresh dichloromethane. Finally, the dissolved carboxylic acids were transferred to a 2-mL vial and stored at –20°C until derivatization for GC-LRMS analysis.

Three of the water samples that were in contact with petroleum (oil field produced water 1, oil field produced water 2 and oil storage tank water) were filtered through glass wool to remove particles and extracted at pH 12 with dichloromethane to remove oil residues. In this procedure, 150 mL – 250 mL of filtered sample were adjusted to pH 12 with 10 % (g v^{-1}) NaOH and then were extracted with three portions of 25 mL of dichloromethane following the extraction the samples were acidified to pH 2 with concentrated HCl and extracted with chloroform as described in this Section 2.2.5.

2.2.6 Solid Phase Extraction Procedure

Jones et al. (2001) developed a procedure for the isolation of aliphatic and naphthenic acids from crude oils by solid phase extraction (SPE) on strong anionic exchange (SAX) column. A similar procedure was applied for the purification of fatty acids and naphthenic acids extracts from 10 water samples and the Merichem naphthenic acids (Table 2.2).

The extracted carboxylic acids were dissolved in 500 µL of hexanes (HPLC grade, Fisher) containing 10% (v v^{-1}) dichloromethane (HPLC grade, Fisher), and then transferred into a 0.5-g BondElut® SAX column (Varian, Lake Forest, CA) previously conditioned with 2 mL of hexanes. After the sample was loaded and the anionic analyte were retained into the SAX sorbent, interferences were removed with 4 mL of hexanes followed by 8 mL of dichloromethane, these two fractions were discarded, and the column was dried by air flush. Finally, the carboxylic acids were eluted from the SAX column with 6 mL of diethyl ether (ACS reagent grade, Fisher) containing 2% (v v^{-1}) of c

formic acid (ACS reagent grade, Fisher), dried under nitrogen, dissolved in fresh dichloromethane, and then transferred to a 2-mL vial for derivatization.

2.2.7 Derivation of Extracted Naphthenic Acids

Clemente et al. (2004) described the derivatization protocol. Briefly, naphthenic acids were derivatized using the *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) reagent, which contains 1% *t*-butyldimethylsilylchloride (Sigma, St. Louis, MO). The naphthenic acids were dissolved in 100 µL of dichloromethane and mixed with 100 µL of MTBSTFA solution. The mixture was then heated at 60°C for 20 min, dried under a stream of nitrogen and the residue was dissolved in 100 µL of fresh dichloromethane until further analysis. Prior to the GC-MS, the extracts were dried and dissolved in 25 µL with dichloromethane for clear water samples and 50 µL or 100 µL for samples that were in contact with petroleum.

2.2.8 Bromination Procedure

Selected samples were brominated to eliminate compounds with carbon-carbon double bonds. The purified and derivatized fatty acids and naphthenic acids from 10 water samples and the Merichem naphthenic acids (Table 2.2) were brominated according to the procedure reported by Hardas et al. (1999). A sample containing the derivatized acids was dried under nitrogen, dissolved in 1 mL of redistilled carbon tetrachloride (ACS grade, Fisher), and transferred to a 5-mL reaction container. Then, 2 mL of carbon tetrachloride with 1% ($v\ v^{-1}$) of bromine (ACS grade, EMD, Darmstadt, Germany) were added to the reaction and the resulting solution was stirred at room temperature in the dark for 2 min. Any excess bromine was removed by reacting with

100 µL of redistilled 2-pentene (ACS grade, Aldrich, St. Louis, MO) until the brownish color disappeared. Finally, the solvent and excess 2-pentene were removed by evaporation under a stream of nitrogen; the residue was dissolved in dichloromethane, and transferred to a 2-mL vial for GC-MS analysis.

2.2.9 GC-Low Resolution MS (GC-LRMS) Analyses

Derivatized residues were typically dissolved in 25 µL, 50 µL or 100 µL of dichloromethane, depending on the expected naphthenic acids concentration and 2 µL of this solution were injected into a Varian Vista 6000 gas chromatograph. A ZB-5 capillary column (Phenomenex®, Torance, CA) (30 m, 0.25 mm ID) with a 0.25 µm film thickness was employed. Helium was used as carrier gas and was set to maintain column pressure at 68.9 kPa. Column temperature was first set at 100°C for 3 min and then programmed from 100 °C to 300°C at a rate of 8°C min^{-1}, with a 12-min hold at 300°C. The GC-MS transfer line was at 290°C and the VG 7070E magnetic sector mass spectrometer was operated in electron impact ionization mode to obtain mass spectra. The scan rate was 1.2 scans s^{-1} and mass scan range was *m/z* 150 to 500. A solvent delay of 180 s was used and the mass spectral information was acquired using the Mass Spec Data System for Windows version 14.0c. RICs were obtained for nominal *m/z* values of 265, 267, and 279.

2.2.10 GC-High Resolution MS (GC-HRMS) Analyses

High Resolution analyses were performed on an Agilent 6890 GC coupled to a Kratos MS50 mass spectrometer in electron impact ionization mode (Department of Chemistry, University of Alberta, Canada). The GC column and the GC conditions were the same as given in Section 2.2.9. Samples (2 µL) of derivatized naphthenic acids were injected in a pulsed splitless mode (2 kPa for 2 min). The MS system was tuned to a minimum resolution of 10,000 (10% valley) using perfluorokerosene as reference gas and lock mass. Acquisition was performed in selected ion monitoring (SIM) mode for the ion with the exact mass of 267.1780 that correspond to a derivatized naphthenic acids with the formula $C_{15}H_{27}SiO_2$, which is the most abundant of the three ions selected to detect naphthenic acids.

2.3 Results and Discussion

2.3.1 Choosing Characteristic Ions for RICs

Given the vast array of compounds with different carbon and Z numbers in any naphthenic acids preparation, it was presumed that certain values of n and Z would be common and abundant in many naphthenic acids samples. GC-LRMS data from 34 naphthenic acids samples were examined to determine which ions were commonly present. Table 2.1 summarizes the abundant ions in five commercial naphthenic acids preparations, in naphthenic acids extracted from 10 samples of oil sands process-affected waters, in naphthenic acids extracted from nine samples of surface waters impacted by process-affected waters, and in naphthenic acids extracted from 10 oil sands ore samples. The selected ions corresponded to naphthenic acids with C_{13} $Z = -4$, C_{13} $Z = -6$ and C_{14} $Z = -6$, giving molecular formula of $C_{13}H_{22}O_2$, $C_{13}H_{20}O_2$, and $C_{14}H_{22}O_2$, respectively. The sums of their abundances show that these three ions account for 6 to 16% of the ions in the commercial preparations (Table 2.1), 5 to 18% in the naphthenic acids from the process-affected waters, 9 to 21% in the naphthenic acids from the surface waters, and 3 to 19% in the naphthenic acids from the oil sands ore samples. Another commonly observed abundant ion in many samples from the oil sands waters and ores corresponds to C14 Z = -4. However, this ion (with nominal *m/z* = 281) was not chosen as one of the ions used for RICs because Clemente and Fedorak (2004) demonstrated that a compound which bleeds from the GC column used for these analyses produces an ion with *m/z* = 281. Thus, the abundance of this ion may be an artifact of the analytical method, and *m/z* = 281 cannot be considered specific for naphthenic acids.

The choices of ions corresponding to C_{13} Z = -4, C_{13} Z = -6, and C_{14} Z = -6 are quite selective for naphthenic acids because naturally occurring fatty acids with 13 carbon atoms and two (C_{13} *Z* = -4) or three unsaturations (C_{13} *Z* = -6) or with 14 carbon atoms with three unsaturations (C_{14} Z = -6) are likely very rare. Therefore, RICs for nominal masses of 265, 267, and 279 should be very specific for the *tert*-butyldimethylsilyl esters of naphthenic acids with formula $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$, and $C_{14}H_{22}O_2$, respectively.

Figure 2.1A shows an example of the *tert*-butyldimethylsilyl ester of a naphthenic acid and the major fragmentation product $C_{15}H_{27}O_2Si^+$ with nominal *m/z* = 267. Based on tabulated masses (St. John et al. 1998, Holowenko et al. 2002) the presence of this ion indicates the presence of naphthenic acids with formula $C_{13}H_{22}O_2$ (C_{13} *Z* = -4).

Scrutinizing published data showed that compounds with molecular formulae $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$, and $C_{14}H_{22}O_2$ (C_{13} *Z* = -6, C_{13} *Z* = -4, and C_{14} *Z* = -6, respectively) have been found in other studies using various analytical methods. For example, using GC-LRMS, St. John et al. (1998) found compounds with these three molecular formulae in an unspecified commercial naphthenic acids preparation.

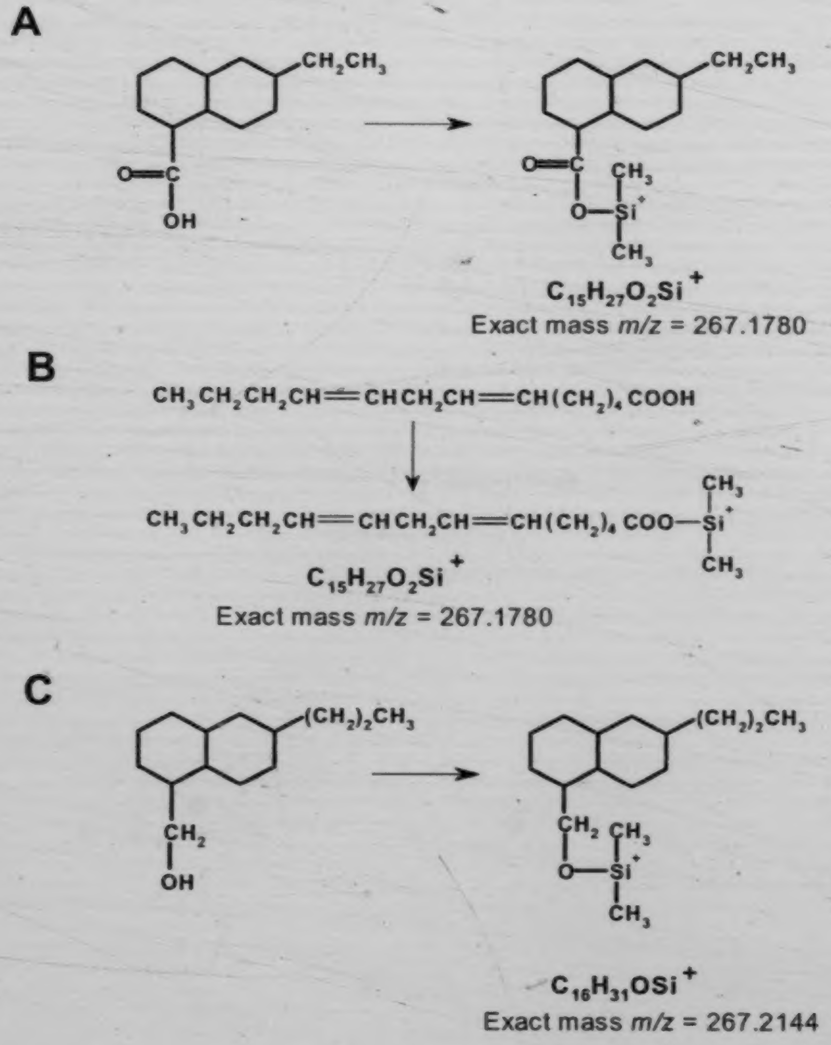

A
CH₂CH₃
O=C
OH
CH₃
O—Si⁺
CH₃
C₁₅H₂₇O₂Si⁺
Exact mass m/z = 267.1780
B
CH₃CH₂CH₂CH=CHCH₂CH=CH(CH₂)₄COOH
CH₃CH₂CH₂CH=CHCH₂CH=CH(CH₂)₄COO—Si⁺
C₁₅H₂₇O₂Si⁺
Exact mass m/z = 267.1780
C
(CH₂)₂CH₃
CH₂
OH
C₁₆H₃₁OSi⁺
Exact mass m/z = 267.2144

Figure 2.1Three compounds that would yield *tert*-butyldimethylsilyl esters when reacted with MTBSTFA. Each would give a nominal mass fragments of *m/z* = 267 when analyzed by GC-LRMS, and would be assigned as a naphthenic acid $C_{13}H_{22}O_2$ based on nominal mass. An example of (A) a naphthenic acid, (B) a hypothetical di-unsaturated carboxylic acid, and (C) a hypothetical alcohol.

Using atmospheric pressure chemical ionization mass spectrometry, Lo et al. (2006) also found compounds with these three molecular formulae in oil sands tailings water. Similarly, ions corresponding to these three molecular formulae were found in naphthenic acids extracted from a petroleum refinery effluent by Wong et al. (1996) who analyzed the extract using fast ion bombardment mass spectrometry. Likewise, ions corresponding to naphthenic acids with formulae $C_{13}H_{20}O_2$, and $C_{13}H_{22}O_2$, (C_{13} $Z = -6$, and C_{13} $Z = -4$, respectively) were detected in an extract of a refinery wastewater analyzed by fluoride ion chemical mass spectrometry (Dzidic et al. 1988). Thus, there is ample evidence that the ions corresponding to C_{13} $Z = -6$, C_{13} $Z = -4$, and C_{14} $Z = -6$ are good indicators of the presence of naphthenic acids. The nominal *m/z* values of 265, 267, and 279 were therefore chosen for RICs to detect the *tert*-butyldimethylsilyl esters of naphthenic acids in a variety of water samples.

2.3.2 RICs from GC-LRMS Analyses of Merichem Naphthenic Acids

An aqueous solution of Merichem acids (100 mg L^{-1}) was extracted, derivatized and analyzed by GC-LRMS. The total ion chromatogram (TIC) and the RICs of the extracted Merichem acids are shown in Figure 2.2. The TIC chromatogram (Figure 2.2A) yielded the characteristic hump that occurs when naphthenic acids are analyzed by GC (Jones et al. 2001, Scott et al. 2005). The RICs for nominal *m/z* values of 265, 267, and 279 (Figure 2B, 2C and 2D, respectively) also yielded humps with retention times from approximately 15 to 23 min. These three ions correspond to the major fragments from the *tert*-butyldimethylsilyl esters of naphthenic acids with formula $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$, and $C_{14}H_{22}O_2$, respectively. Of course, many different isomers are represented by each formula. For example, given a simpler formula $C_{10}H_{18}O_2$ (where $Z = -2$), and assuming that each isomer has one 6-membered ring, 37 different carboxylic acids can fit these criteria (Clemente and Fedorak 2005). Because the GC cannot separate the individual isomers, the RICs would be expected to yield humps. The results in Figure 2.2 clearly illustrate that ions with nominal *m/z* values of 265, 267, and 279 are indicators of the presence of naphthenic acids.

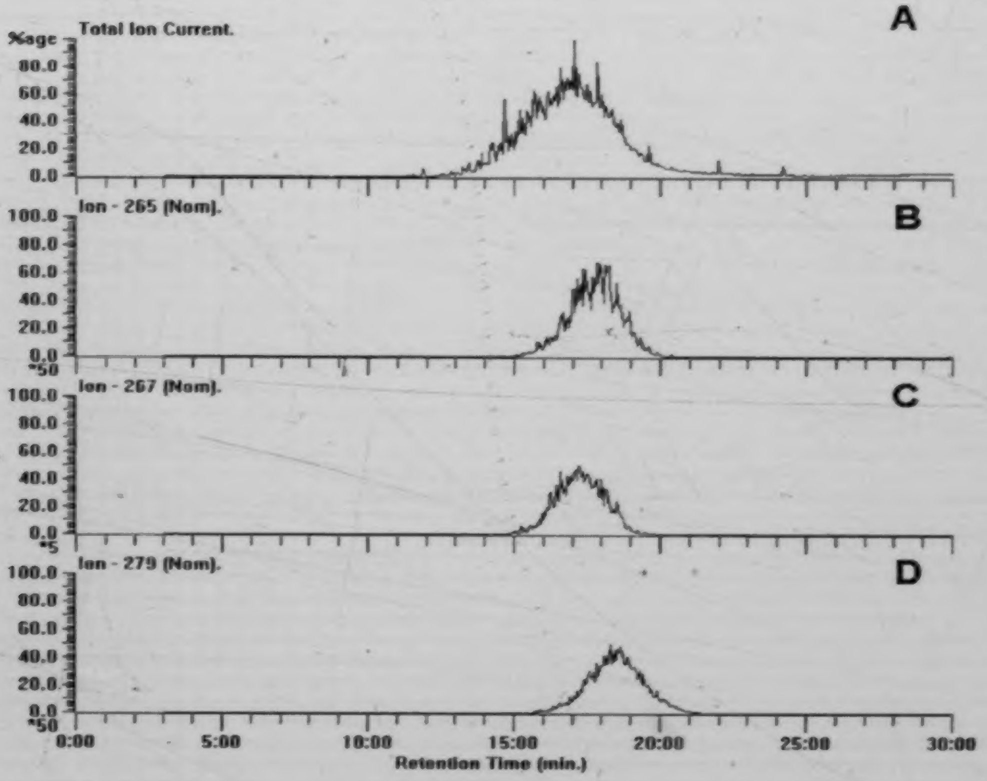


Figure 2.2 (A) TIC chromatogram from the GC-LRMS analysis after extraction of a solution of 100 mg Merichem acids L^{-1}. RICs for (B) $m/z = 265$ (C_{13}, $Z = -6$); (C) $m/z = 267$ (C_{13}, $Z = -4$) and (D) $m/z = 279$ (C_{14}, $Z = -6$).

2.3.3 Detection of Naphthenic Acids in Water that Contacted Oil Sands Ore

After 24 h of shaking, each of the three doses of oil sands ore (3,000 mg L^{-1}, 600 mg L^{-1}, and 100 mg L^{-1}) in North Saskatchewan River water was extracted, derivatized and analyzed by GC-LRMS. The RICs for the nominal masses of 265, 267, and 279 showed humps with retention times from approximately 15 to 23 min, consistent with the presence of naphthenic acids (data not shown). No hump was observed when an extract of North Saskatchewan River water analyzed in the same manner.

Clemente and Fedorak (2005) reported that the average naphthenic acids content of seven oil sands ore samples was 200 mg kg^{-1} ore. Thus, if all of the naphthenic acids were extracted from the oil sands ore in this simple shaking batch experiment, the naphthenic acids concentrations would have been about 0.6 mg L^{-1}, 0.1 mg L^{-1}, and 0.01 mg L^{-1} in the water samples with the three different doses of ore. These results clearly indicate that naphthenic acids can be extracted from oil sands ore that is in contact with river water.

2.3.4 Sensitivity of the GC-LRMS Method for Naphthenic Acids

To estimate the sensitivity of the GC-LRMS, 1-L samples of North Saskatchewan River water were spiked with 100, 10 or 1 μg of Merichem naphthenic acids. After extraction and derivatization, these samples were analyzed by GC-LRMS. Figure 2.3 shows the TIC and RIC ($m/z = 267$) from the analysis of the sample that contained 10 μg erichem naphthenic acids L^{-1} river water (equivalent to 10 parts per billion). The hump from the naphthenic acids is easily discernable in Figure 2.3B, with retention time from 16 to 21 min. Again, no hump was observed when an extract of North Saskatchewan

River water analyzed in the same manner (data not shown). Therefore, the hump in Figure 2.3B was due to the added naphthenic acids. Using this GC-LRMS method, naphthenic acids could not be detected from the 1-L water sample that was spiked with 1-µg Merichem naphthenic acids. Based on these results from the extraction of 1 L of water, the detection limit of this method is approximate 10 µg naphthenic acids L^{-1}.

Scott et al. (2005) demonstrated that there are considerable differences between the TIC chromatograms of the naphthenic acids from commercial sources and those from oil sands tailings waters. To determine if the sensitivity of the GC-LRMS method was similar for naphthenic acids from oil sands tailings waters, a 1-L sample of North Saskatchewan River water was spiked with 10 µg of concentrated naphthenic acids extracted from Syncrude tailings water. The RIC for (*m/z* 267) yielded a hump of naphthenic acids with retention times from approximately 16 to 20 min (data not shown). These results indicate that the sensitivity of the GC-LRMS method is similar for naphthenic acids from a commercial source or from oil sands process water.

The ability to detect naphthenic acids in the river water that was spiked with 10 µg naphthenic acids L^{-1} is remarkable. Few other researchers have described an analytical method for detecting naphthenic acids at this low concentration. For example, the HPLC method of Yen et al. (2004) has a detection limit of about 5 mg L^{-1}, and various mass spectrometry methods have used oil sands naphthenic acids at concentrations of 1 mg L^{-1} (Gabryelski and Froese 2003, Barrow et al. 2004). Only Headley et al. (2002) have described a method for naphthenic acids analysis using negative ion electrospray mass spectrometry, which also has a detection limit of 10 µg L^{-1}.

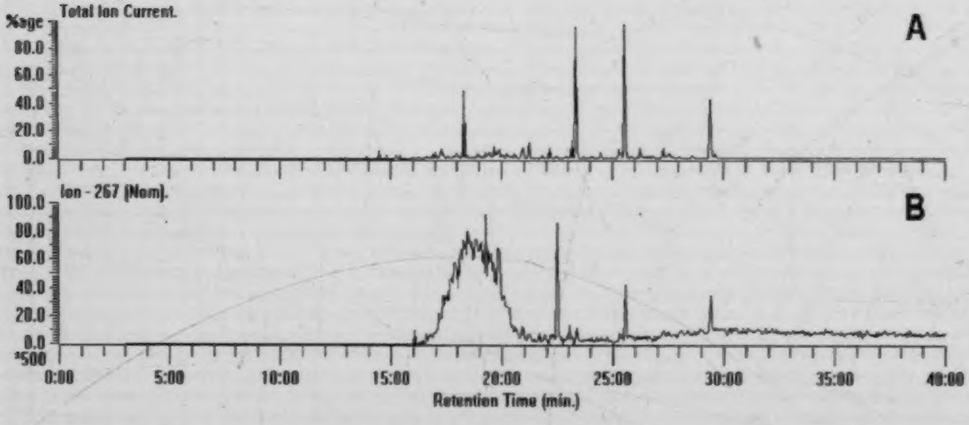


Figure 2.3 (A) TIC chromatogram from the GC-LRMS analysis after extraction of a solution of 10 µg Merichem acids L^{-1} of North Saskatchewan River water. (B) RIC for *m/z* = 267 showing the characteristic hump of naphthenic acids between 15 to 20 min retention time.

2.3.5 Detection of Naphthenic Acids in Water Samples from Various Sources

Water samples from 23 different locations were collected and analyzed for naphthenic acids using the GC-LRMS method. The results from the RICs of the ions corresponding to $C_{15}H_{27}O_2Si^+$ (*m/z* = 267) are summarized in Tables 2.2 and 2.3, which also contains data from the analyses of Merichem naphthenic acids (sample 14).

Eight surface water and five ground water samples were obtained from a variety of sources (samples 1 to 13, Table 2.2), many of which were remote from oil sands or petroleum producing activities (e.g. Waverly Creek, North Thompson River, Swift Creek, Moose Lake, Gregg Lake, North Saskatchewan River, and a pond east of Edmonton). RICs (*m/z* 267) of the derivatized extracts of all of these samples showed no evidence of naphthenic acids by GC-LRMS (Table 2.2). Thus, no further analyses were conducted with the 13 water samples summarized in Table 2.2.

Table 2.2 Summary of the detection of naphthenic acids from RICs and SIM results in a variety of water samples used in this study.

Sample No.	Samples and Locations[a]	Color mg L^{-1}	Naphthenic acids detected by the presence of ions corresponding to $C_{15}H_{27}O_2Si^+$ GC-LRMS RIC $m/z = 267$
	Surface waters		
1	Waverly Creek, Thunder Bay ON	760	–
2	North Thompson River BC	4	–
3	Swiff Creek, Valemont BC	2	–
4	Moose Lake BC	3	–
5	Gregg Lake AB	17	–
6	N. Saskatchewan River, Edmonton AB	75	–
7	Athabasca River, Fort McMurray AB	160	–
8	Athabasca River, near Albian Sands	40	–
	Ground Waters		
9	Artisan spring, Hinton AB	12	–
10	Public well Gregg Lake AB	14	–
11	Domestic well 1, 30 km southwest of Ottawa (ON)	2	–
12	Domestic well 2, 30 km southwest of Ottawa (ON)	4	–
13	Domestic well 3, 50 km east of Edmonton AB	17	–

[a] Canadian provinces in which samples were collected AB Alberta, BC British Columbia, ON Ontario.

Because naphthenic acids are found in petroleum, five samples of waters that had direct contact with petroleum (samples 15 to 19, Table 2.3) were analyzed. One from an oil sands tailing pond, one was from a petroleum refinery wastewater, two were produced waters from oil fields that used water flooding to enhance oil recovery, and one was water that settled to the bottom of a crude oil storage tank. RICs (*m/z* 267) of the derivatized extracts from these waters detected naphthenic acids in each sample as shown in Table 2.3 column GC-LRMS No Br_2 (samples 15 to 19). These results are consistent with other studies, which have reported that naphthenic acids are present in petroleum refinery wastewaters (Dzidic et al. 1988, Dorn 1992, Wong et al. 1996) and in produced waters (Havre et al. 2003).

The Athabasca River, which cuts through the oil sands deposits, was sampled from three locations. One was in the city of Fort McMurray (sample 7, Table 2.2), before the river reaches the exposed oil sands. No naphthenic acids were detected in this sample. As the Athabasca River flows away from the city, it first passes Suncor, then Syncrude, then Albian Sands oil sands operations. Based on the results from the RICs, naphthenic acids were not detected in the river water sample taken near the Albian Sands operation (sample 8, Table 2.2). However, these acids were detected in the sample taken near the Syncrude operation (sample 20, Table 2.3, column GC-LRMS No Br_2) in agreement with results reported by MacKinnon et al. (2005) based on FTIR analysis. At Syncrude, water is pumped from the Athabasca River into Mildred Lake Reservoir, which serves as a source of water for Syncrude's extractions. RICs showed that naphthenic acids were also detected in the reservoir sample (sample 21, Table 2.3, column GC-LRMS No Br_2).

Schramm et al. (2000) also used the FTIR method to survey naphthenic acids concentrations in eleven Athabasca River water samples collected from about 100 km upstream of Fort McMurray to the delta of Lake Athabasca. They reported finding naphthenic acids in all of the samples, with concentrations ranging from about 100 to 900 μg L^{-1}. However, based on the more selective GC-MS method, the RICs for *m/z* 267 showed that only one of the three Athabasca River water samples contained naphthenic acids at concentration greater than 10 μg L^{-1} approximately. This disagreement was most likely due to the lack of specificity of the FTIR method.

A highly colored pond water (sample 22, Table 2.3), which was distant from the oil sands, was also examined for the presence of naphthenic acids. The RIC of this pond water extract showed a hump with retention time from 17 to 28 min, which was much broader than typical for *m/z* 267. Further analyses of this sample are discussed in Section 2.3.6.

There is increasing interest in the possibility of groundwater transporting dissolved components (including naphthenic acids) in oil sands process-affected waters away from the tailing ponds (Gervais 2004, MacKinnon et al. 2004, Oiffer 2006). Thus, seven groundwater samples were collected from locations that were distant from the oil sands (samples 9 to 13, Table 2.2 and samples 23 and 24, Table 2.3). It was anticipated that GC-LRMS analyses of the extracts of these samples were expected not to show any signs of naphthenic acids. This was true for five of the samples (samples 9 to 13, Table 2.2), but surprisingly two of these samples from domestic wells on acreages near Edmonton (samples 23 and 24, Table 2.3) showed evidence of naphthenic acids based on the RICs for *m/z* = 267, 265, and 279.

Table 2.3 Summary of the detection of naphthenic acids from RICs and SIM results in a variety of water samples used in this study.

			Naphthenic acids detected by presence of ions corresponding to $C_{15}H_{27}O_2Si^+$			
			GC-LRMS RIC *m/z* =267		GC-HRMS RIC *m/z* =267	
Sample No.	Samples and Locations [a]	Color mg L^{-1}	No Br_2	Br_2	No Br_2	Br_2
	Petroleum Sources					
14	Merichem refined naphthenic acids	NA	+	+	+	+
15	Oil sands tailings water (WIP)		+	+	+	+
16	Petroleum refinery wastewater, Edmonton AB	250	+	+	+	–
17	Oilfield produced water 1, Stettler AB	70	+	+		
18	Oilfield produced water 2, Stettler AB	30	+	+		–
19	Oil storage tank water, Edmonton AB	430	+	–	–	
	Surface waters					
20	Athabasca River, near Syncrude	55	+	+	+	+
21	Mildred Lake Reservoir at Syncrude	40	+	+	+	+
22	Pond 50 km east of Edmonton AB	480	± [b]	–	–	–
	Ground Waters					
23	Domestic well 4, 20 km east of Edmonton AB	48	+	+	+	+
24	Domestic well 5, 40 km northwest of Edmonton AB	19	+	+	+	+

[a] Canadian provinces in which samples were collected AB Alberta, BC British Columbia, ON Ontario, [b] Reconstructed ion chromatogram (*m/z* 267) with a hump with retention time between 17 to 28 min.

2.3.6 Addressing the Apparent Anomalous Results from GC-LRMS analyses

The apparent presence of naphthenic acids in the pond east of Edmonton (sample 22, Table 2.3) and in two domestic well waters near Edmonton (samples 23 and 24, Table 2.3) were considered to be anomalies because there was no indication that these waters had been in contact with naphthenic acids-containing materials. Thus, further analyses were conducted in these samples in order to attempt to disprove the presence of naphthenic acids in these three water samples.

As with all mass spectrometry methods used to characterize naphthenic acids, data analysis of results from the GC-LRMS assigns ions of a given *m/z* value to appropriate *n* and *Z* values, which in turn indicates the presence of isomers of naphthenic acids with formula $C_nH_{2n+Z}O_2$. For example, when GC-LRMS analysis the *tert*-butyldimethylsilyl esters of naphthenic acids yields ions with *m/z* values of 265, 267, and 279, it is assumed that this ions correspond to naphthenic acids with formula $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$, and $C_{14}H_{22}O_2$, respectively (St. John et al. 1998, Clemente and Fedorak 2005). However, it is possible that some compounds in the extracts of the anomalous samples simply are not naphthenic acids, yet yield ions of *m/z* = 265, 267, and 279, and these were mistakenly considered to be naphthenic acids.

2.3.7 Use of SPE Columns to Clean up Naphthenic Acids Fraction

Extracts from the 11 samples that showed the presence of naphthenic acids based on RICs for *m/z* = 267 (scored "+" or "±" in Table 2.3, column GC-LRMS No Br_2) were fractionated through SAX SPE columns. The fraction that elutes with 2% formic acid in ether is known to contain carboxylic acids, such as naphthenic acids (Jones et al. 2001).

After derivatizing the residues in these fractionated samples, each sample was analyzed by GC-LRMS. Figure 2.4 illustrated the marked change in the TIC chromatograms from the extracts of the oil storage tank water before (Figure 2.4A) and after (Figure 2.4C) fractionation through SAX SPE column. Similarly, passage of the sample through the SAX SPE column altered the RIC for *m/z* = 267 (Figure 2.4B and D). However, the hump between 15 and 23 min, that is presumably due to naphthenic acids more obvious (Figure 2.4D) after passage through the SAX SPE. All of the 11 samples, including the three of the anomalous samples (22, 23, and 24, Table 2.3), that were fractionated and analyzed by GC-LRMS gave humps in the RICs for *m/z* = 265, 267, and 279 (data not shown). Thus, the results from SAX SPE column fractionation did not disprove the presence of naphthenic acids in these three anomalous samples.

Fulvic acids are known to impart color to natural waters, and the pond water and domestic well waters (22, 23, and 24, Table 2.3) had a yellow color. Thus, samples of reference fulvic acids dissolved in North Saskatchewan River water (20 mg L^{-1}) were extracted, derivatized and analyzed by GC-LRMS to determine if these acids might be responsible for the humps in the RICs for ions of *m/z* = 265, 267, and 279. Analysis of this extract, prior to passage through a SAX SPE column showed individual peaks with these nominal masses, but no humps were observed (data not shown). Most of these peaks were absent when the extract was passed through a SAX SPE column. Therefore, it is very unlikely that fulvic acids were responsible for the hump of ions with *m/z* = 265, 267, and 279 detected in these three samples.

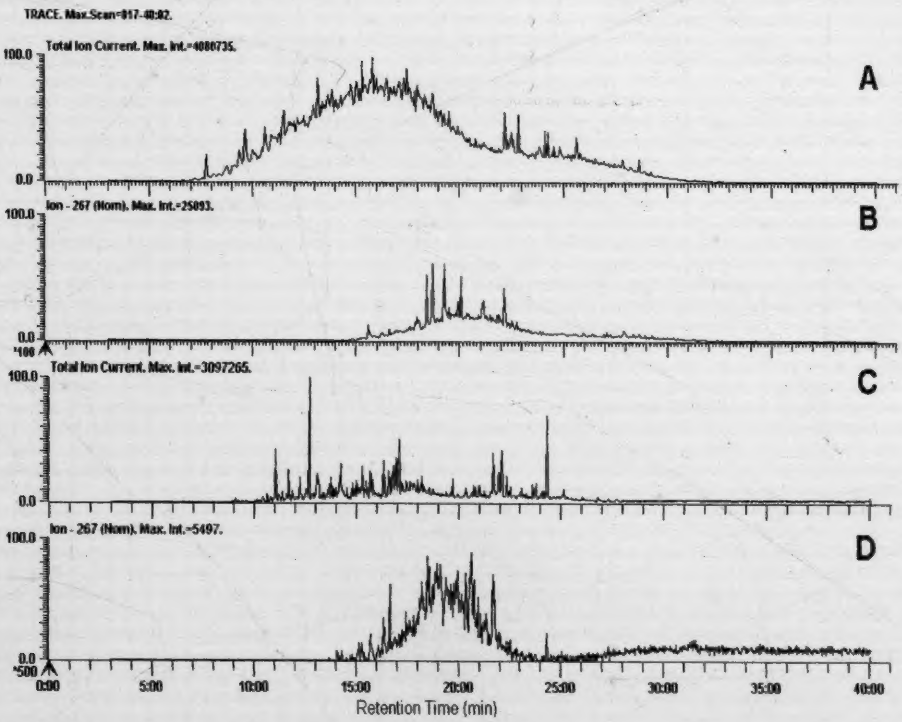


Figure 2.4 GC-LRMS analyses of the extract of water from an oil storage tank. TIC chromatograms before (A) and after (C) clean up through a SAX SPE column, RICs for *m/z* = 267 before (B) and after (D) clean up through a SAX SPE column.

2.3.8 Bromination of Naphthenic Acids-Containing Fractions

Naphthenic acids are generally considered to be predominantly alkyl-substituted cycloaliphatic carboxylic acids (Brient et al. 1995) and in general, unsaturated acids are not considered to be major components of naphthenic acids (Brient et al. 1995). A hypothetical di-unsaturated fatty acids, as shown in Figure 2.1B, fits the formula for naphthenic acids (C_{13} $Z = -4$). The formation and GC-LRMS analysis of the *tert*-butyldimethylsilyl ester of this di-unsaturated carboxylic acid would be incorrectly assigned as naphthenic acids because of the appearance of ions with *m/z* 267. Thus, to eliminate the possible incorrect assignment of the di-unsaturated acids as naphthenic acids after passage through the SAX SPE column, selected *tert*-butyldimethyl silylated extracts from various sources were treated with Br_2. Bromination across the two double bonds of di-unsaturated fatty acids would result in a 320 Dalton increase in the molecular mass of the brominated fatty acids, and the newly formed tetrabrominated compounds would not appear in the hump of compounds with *m/z* = 267 from GC-LRMS analysis. After bromination, the characteristic humps were found in all of the samples except those from the oil storage tank and the pond waters (samples 19 and 22, Table 2.3, column GC-LRMS Br_2), indicating that naphthenic acids were not present in these two samples. However, humps were still found in the extracts from domestic well waters (samples 23 and 24, Table 2.3, column GC-LRMS Br_2). Thus, I could not disprove the presence of naphthenic acids in these two well water samples. Figures 2.5A and 2.5B compare the RICs of sample 23, before and after bromination.

2.3.9 GC-HRMS

Nine samples (14, 15, 16, 18, 20, 21, 22, 23 and 24, Table 2.3) that showed the presence of naphthenic acids by GC-LRMS were chosen for GC-HRMS analyses using SIM with the exact mass of 267.1780, corresponding $C_{15}H_{27}O_2Si^+$, from the *tert*-butyldimethylsilyl esters of naphthenic acids with formula $C_{13}H_{22}O_2$ (Figure 2.1A). The SIM chromatograms of the Merichem acids, the tailings water sample and the petroleum refinery wastewater showed clearly defined humps that eluted with retention times from 15 to 20 min, consistent with the presence of naphthenic acids (scored + in Table 2.3, column GC-HRMS SIM No Br_2). These were similar in appearance to the RICs shown in

Figures 2.2B and 2.3B. In contrast, the SIM chromatograms of the derivatized extract from the oil storage tank water showed no distinct hump that is characteristic of naphthenic acids (sample 19, Table 2.3, column GC-HRMS SIM No Br_2). This result was consistent with the result from the GC-LRMS analysis of the brominated sample from the oil storage tank water (sample 19, Table 2.3, column GC-LRMS RIC after Br_2).

Based on the SIM analyses, naphthenic acids were also present in the Athabasca River water taken near Syncrude (sample 20, Table 2.3), in the Mildred Lake reservoir at Syncrude (sample 21, Table 2.3), and in the water from domestic wells (samples 23 and 24, Table 2.3, column GC-HRMS No Br_2). However, the ion corresponding to $C_{15}H_{27}O_2Si^+$ was not detected in the SIM analysis of the pond water (sample 22, Table 2.3 column GC-HRMS No Br_2), which is in agreement with the results from the GC-LRMS analysis of the brominated sample of pond water (Table 2.3).

The humps detected in the RIC $m/z = 267$ from GC-LRMS may correspond to compounds that are not naphthenic acids. For example, an alcohol with formula $C_{14}H_{26}O$ shown in Figure 2.1C would react with MTBSTFA to yield a *tert*-butyldimethylsilyl ether that would fragment to produce an ion with nominal mass 267. Thus, based on the GC-LRMS method, this alcohol would be incorrectly assigned as a naphthenic acid with the molecular formula $C_{13}H_{22}O_2$. To assess whether this misassignment might be happening, selected water samples (samples 14, 15, 16, 19, 20, 22, 23, and 24, Table 2.3) were analyzed by SIM GC-HRMS for exact mass 267.2144 that corresponds to $C_{16}H_{31}OSi^+$, (Figure 2.1C). No hump corresponding to the exact mass 267.2144 was detected in the extracts from domestic well samples 23 and 24 (Table 2.3), providing further evidence that compounds which are indistinguishable from naphthenic acids were present in these two well water samples. Furthermore, no humps corresponding to this exact mass were found in the extracts from the pond water (sample 22, Table 2.3), from the Athabasca River water sample taken near Syncrude (sample 20, Table 2.3), or from the oil storage tank (sample 19, Table 2.3). However, ions with exact mass 267.2144 were found in the Merichem acids (sample 14) and in the extracts from the oil sands tailings water (sample 15, Table 2.3) and from petroleum refinery wastewater (sample 16, Table 2.3).

Subsequently, nine brominated samples (samples 14 to 16, 18, and 20 to 24, Table 2.3, column GC-HRMS after Br_2) were analyzed by SIM with the exact mass of 267.1780, corresponding $C_{15}H_{27}O_2Si^+$, from the *tert*-butyldimethylsilyl esters of naphthenic acids with formula $C_{13}H_{22}O_2$ (Figure 2.1A). This ion was detected in the Merichem acids (sample 14, Table 2.3), in the extracted oil sands naphthenic acids (sample 15, Table 2.3), and in samples from the Athabasca River near Syncrude (sample 20, Table 2.3) and in the Mildred Lake Reservoir at Syncrude (sample 21, Table 2.3). However, SIM did not detect naphthenic acids in the brominated extracts from the petroleum refinery wastewater (sample 16, Table 2.3) or from the oilfield produced water 2 (sample 18, Table 2.3). These last two observations are in contrast to the results from the GC-LRMS analyses of the brominated extracts. Because of the higher specificity of the SIM method, these results are more accurate as SIM is base on exact masses rather than nominal masses, thus the results from sample 16 and 18 (Table 2.3) are negative for the presence of naphthenic acids. Finally, SIM detected naphthenic acids in the brominated extracts from domestic wells samples 23 and 24 (Table 2.3), consistent with all of the other analyses of these two samples. Figures 2.5C and 2.5D compare the SIM chromatograms of sample 23, before and after bromination. Thus, these two well waters either contain naphthenic acids, or they contain materials that are indistinguishable from naphthenic acids by the variety of analytical methods used herein. This is the first report of the possible presence of naphthenic acids in domestic well waters.

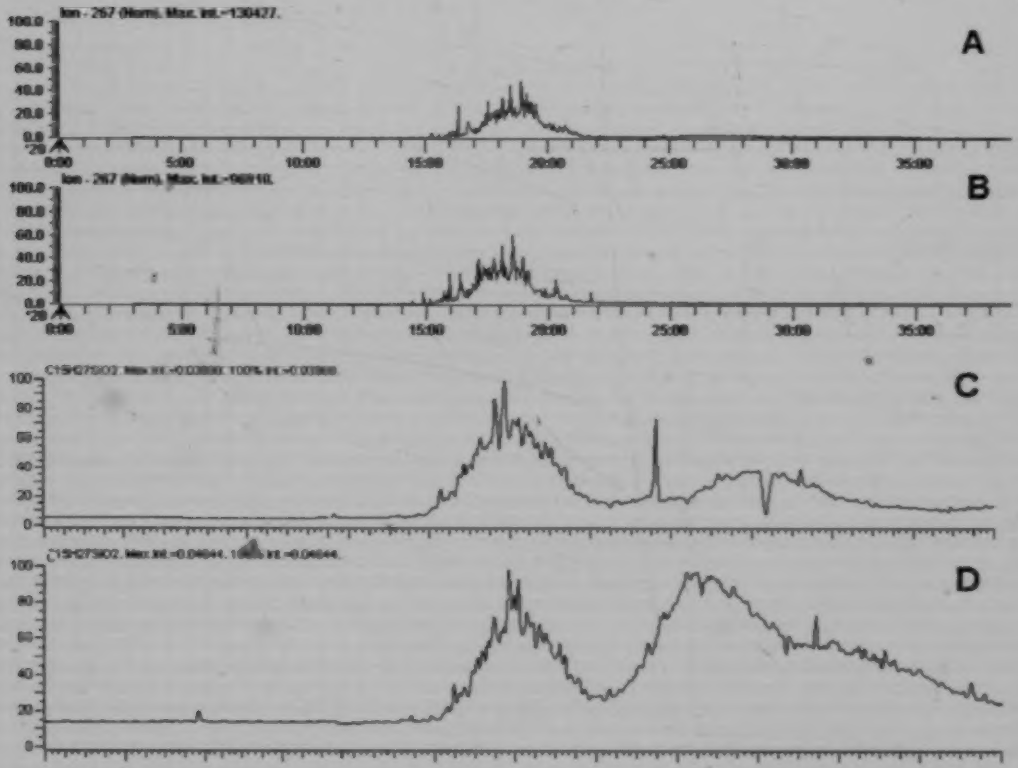


Figure 2.5 GC-MS analyses of the extract of water from domestic well sample 23 (Table 2.3) RICs (m/z = 267) before (A) and after (B) bromination, SIM chromatograms (m/z = 267.1780) before (C) and after (D) bromination.

2.4 Conclusions

The qualitative and quantitative analyses of naphthenic acids are major challenges. This investigation provides procedures for the detection of naphthenic acids in water samples. It is based on using GC-MS to detect ions that correspond to the *tert*-butyldimethylsilyl esters of naphthenic acids with the following carbons and *Z* numbers: C_{13} $Z = -4$, C_{13} $Z = -6$, and C_{14} $Z = -6$. In addition, the results presented in Table 2.3 column GC-LRMS no Br_2 are based on duplicate sample analyses, which gave the same results.

Typically, one of the most abundant ions correspond to C_{13} $Z = -4$, and this method key on these ions whose *tert*-butyldimethylsilyl esters fragmented to produce $C_{15}H_{27}O_2Si^+$ ($m/z = 267$). Using SAX SPE column cleanup and bromination improved the specificity of the method. In most cases, the results from GC-LRMS and SIM monitoring with GC-HRMS were the same. However, in a few cases (those of the oilfield produced waters), the GC-LRMS method indicated the presence of naphthenic acids, whereas the GC-HRMS method indicated the absence of naphthenic acids. Thus, in the latter case, the samples were reported as negative for the presence of naphthenic acids because SIM analyses are based on exact mass rather than nominal mass, so they are more accurate.

Although this method can detect as little as 10 μg naphthenic acids L^{-1} in a 1-L water sample, it is strictly a qualitative method. More work is required to make this a "quantitative" method, and the results would depend on the composition of the naphthenic acids mixture in a given sample.

Despite some of the limitations given above, the combination of these extraction, clean-up, derivation, bromination and GC-HRMS methods provide a useful means for specifically detecting naphthenic acids in waters. Variations of these methods were used to detect naphthenic acids in plant and animal tissues and these results are the topic of the later chapters in this thesis.

2.5 Literature Cited

Barrow, M.P., Headley, J.V., Peru, K.M. and Derrick, P.J. 2004. Fourier transform ion cyclotron resonance mass spectrometry of principle components of oilsands naphthenic acids. Journal of Chromatography A, 1058: 51-59.

Brient, J.A., Wessner, P.J. and Doly, M.N.,1995. Naphthenic acids. In: Kroschwitz, J.I. (Ed.), In: Encyclopedia of Chemical Technology, 4th ed. Vol. 16. John Wiley and Sons, New York, NY. pp. 1017-1029.

Clemente, J.S. 2004. The characterization analyses and biodegradation of naphthenic Acids MSc. Thesis. University of Alberta, Edmonton, Canada.

Clemente, J.S. and Fedorak, P.M. 2004. Evaluation of the analyses of *tert*-butyldimethylsilyl derivatives of naphthenic acids by gas chromatography-electron impact mass spectrometry. Journal of Chromatography A, 1047: 117-128.

Clemente, J.S. and Fedorak, P.M. 2005. A review of the occurrence, analyses, toxicity and biodegradation of naphthenic acids. Chemosphere, 60: 585-600.

Clemente, J.S., Prasad, N.G.N., MacKinnon, M.D. and Fedorak, P.M. 2003. A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. Chemosphere, 50: 1265-1274.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology, 38: 1009-1016.

Cuthbert, I.D. and del Giorgio P. 1992. Toward a standard method of measuring color in fresh water. Limnology and Oceanography, 37: 1319-1326.

Dorn, P.B. 1992. Case histories – the petroleum refining industry. In D.L. Ford (ed) Toxicity Reduction Evaluation and Control. Water Quality Management Library. Vol. 3. Technomic Publishing Company Inc., Lancaster, PA. pp. 183-223.

Dzidic, I., Somerville, A.C., Raia, J.C. and Hart, H.V., 1988. Determination of naphthenic acids in California crudes and refinery wastewaters by fluoride-ion chemical ionization mass-spectrometry. Analytical Chemistry, 60: 1318-1323.

Fan, T.P. 1991. Characterization of naphthenic acids in petroleum by fast atom bombardment mass spectrometry. Energy and Fuels, 5: 371-375.

Fatoki, O.S. and Vernon, F. 1989. Determination of free fatty acid content of polluted and unpolluted waters. Water Research, 23: 123-125.

Gabryelski, W. and Froese. K.L. 2003. Characterization of naphthenic acids by electrospray ionization high-field asymmetric waveform ion mobility spectrometry mass spectrometry. Analytical Chemistry, 75: 4612-4623.

Gervais, F.J.M. 2004. Fate and transport of naphthenic acids in a glacial aquifer. MSc. Thesis. University of Waterloo, Waterloo, Canada.

Hardas, N.R., Adam, R. and Uden, P.C. 1999. Alkene determination by bromination and gas chromatography with element-selective atomic plasma spectroscopic detection. Journal of Chromatography A, 844: 249-258.

Havre,T.E., Sjoblom, J. and Vindstad, J.E. 2003. Oil/water-partitioning and interfacial behavior of naphthenic acids. Journal of Dispersion Science and Technology, 24: 789-901.

Headley, J.V., Peru, K.M. McMartin, D.W. and Winkler, M. 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. Journal of AOAC International, 85: 182-187.

Holowenko, F.M., MacKinnon, M.D and Fedorak, P.M. 2001. Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research, 35: 2595-2606.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research, 36: 2845-2857.

Jivraj, M.N., MacKinnon, M. and Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Analytical Methods Manual. 4th ed. Research Department, Syncrude Canada Ltd., Edmonton, Canada.

Jones, D.M., Watson, J.S., Meredith, W., Chen, M. and Bennett, B. 2001. Determination of naphthenic acids in crude oils using nonaqueous ion exchange solid-phase extraction. Analytical Chemistry, 73: 703-707.

Lo, C.C, Brownlee, B.G. and Bunce, N.J. 2006. Mass spectrometry and toxicological assays of Athabasca oil sands naphthenic acids. Water Research, 40: 655-664.

MacKinnon, M.D., Kampala, G., Marsh, B., Fedorak, P. and Guigard, S. 2004. Indicators for assessing transport of oil sands process-affected waters. Proceedings of the 4th International Groundwater Quality Conference. Waterloo, Canada. July 2004. IAHS publication 297. pp 71-80.

Murray, K.K., Boyde, R.K., Eberlin, M.N., Langley, G.J., Li, L. and Naito, Y. 2006. IUPAC Standard Definitions of Terms relating to Mass Spectrometry. IUPAC project 2003-056-2-500. http://www.msterms.com/docs/IUPAC_MS_Terms_Second_Draft.pdf

Oiffer, A.A.L. 2006. Integrated solid phase, aqueous phase and numerical investigation of plume geochemistry at an oil sands mining facility. MSc. Thesis. University of Waterloo, Waterloo, Canada.

Rudzinski, W.E., Oehlers, L. and Zhang, Y. 2002. Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy and Fuels. 16: 1178-1185.

Schramm, L.L., Stasiuk, E.N. and MacKinnon, M.D. 2000. Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes. In: Surfactants: Fundamentals and Applications in the Petroleum Industry. Schramm, L.L. (Ed). Cambridge University Press. Cambridge, UK. pp. 365-430.

Scott, A.C., MacKinnon, M.D. and Fedorak, P.M. 2005. Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environmental Science and Technology, 39: 8388-8394.

Seifert, W.K. and Teeter, R.M. 1969. Preparative thin-layer chromatography and high-resolution mass spectrometry of crude oil carboxylic acids. Analytical Chemistry, 41: 786-795.

Seifert, W.K., Teeter, R.M., Howells, W.G. and Cantow, M.J.R. 1969. Analysis of crude oil carboxylic acids after conversion to their corresponding hydrocarbons. Analytical Chemistry, 41: 1638-1647.

St. John, W.P., Righani, J., Green, S.A. and McGinnis, G.B. 1998. Analysis and characterization of naphthenic acids by gas chromatography-electron impact mass spectrometry of *tert*-butyldimethylsilyl derivatives. Journal of Chromatography A, 807: 241-251.

Tissot, B.P. and Welte, D.H. 1978. Petroleum Formation and Occurrence. Springer-Verlag, New York, NY.

Tomczyk, N.A., Winans, R.E., Shinn, J.H. and Robinson, R.C. 2001. On the nature and origin of acidic species in petroleum. 1. Detailed acid type distribution in a California crude oil. Energy and Fuels, 15: 1498-1504.

Vaz de Campos, M.C., ConceiÇão, E.O., Sanchez, P.J.F., Sartori, C.M.P. and Bastos, E.C. 2006. Analysis of *tert*-butyldimethylsilyl derivatives in heavy gas oil from Brazilian naphthenic acids by gas chromatography coupled to mass spectrometry with electron impact ionization. Journal of Chromatography A, 1105: 95-105.

Wong, D.C.L., van Compernolle, R., Nowlin, J.G., O'Neal, D.L. and Johnson, G.M., 1996. Use of supercritical fluid extraction and fast ion bombardment mass spectrometry to identify toxic chemicals from a refinery effluent adsorbed onto granular activated carbon. Chemosphere, 32: 1669-1679.

Yen, T.W., Marsh, W.P., MacKinnon, M.D. and Fedorak, P.M. 2004. Measuring naphthenic acids concentrations in aqueous environmental samples by HPLC. Journal of Chromatography A, 1033: 83-90.

3. Detection of Naphthenic Acids in Plants Tissues by Gas Chromatographic - Mass spectrometry

3.1 Introduction

The Athabasca bitumen and heavy oil deposits are one of the largest oil reserves in the world located in Northeastern Alberta, Canada that produce more than 200 million barrels of light sweet crude oil per year, but with projection to increase the production to supply more than 50% of Canada's oil in near future (MacKinnon 1989, Masliyah 2002). The bitumen at the Athabasca deposits is extracted from the oil sands using the Clark aqueous digestion (Kasperski 1992). This process requires water and sodium hydroxide as conditioning. It is estimated that for each m^3 of oil sand processed, about 3 m^3 of water are required and this generates about 4 m^3 of fluid waste (Clemente et al. 2003). These fluid wastes are called tailings and they consist of sand, clay, water and un-recovered bitumen that are deposited in large tailings ponds (MacKinnon 1989). After settling of the solids in the tailings ponds, clear water is recycle back to the extraction plant and is called process-affected water. Environmental regulations in Alberta, prevent the oil sands companies from discharge any process-affected waters into aquatic ecosystems due to concerns about toxicity, thus the tailings are stored in large ponds until they are reclaimed (Lo et al. 2003).

The tailings waters are characterized by high concentrations of dissolved ions predominantly SO_4^{2-} and Na^+, high pH between 8.5 to 10.15 (Yen et al. 2004) and naphthenic acids, a group of low molecular weight organic acids traced as the major contributors of toxicity in the oil sands process-affected waters (MacKinnon and Boerger 1986, Madill et al. 2001).

Naphthenic acids compromise a complex group of acyclic and cycloaliphatic carboxylic acids that are dissolved into the alkaline tailings as sodium naphthenates. Typically, tailings waters contain 20 to 120 mg of naphthenic acids L^{-1} and these acids are defined by the chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number and Z the homologous family with 0 to -12 (Clemente et al. 2004). Naphthenic acids with $Z= 0$ are believed to be highly branched compounds because straight chain fatty acids are easily biodegraded by indigenous microorganism over time, and as the integer of Z decrease from 0 to -12 the number of ring structures increased and the compounds became more complex with multiple combinations of 5- or 6-mermbered rings (Holowenco et al. 2001). In nature, naphthenic acids are found as typical components of petroleum deposits (Seifert et al. 1969, Fan 1991, Tomczyk et al. 2001) including the Athabasca oil sands ores (Clemente 2004). Laboratory studies suggested that these acids are the result of partial biodegradation by microorganisms (Roques et al. 1994).

Naphthenic acids exert toxic effects on plants. A microcosm study by Crowe et al. (2001) measured the effect of oil sands effluents on cattails and clover plants growing in the presence of high conductivity levels, slightly basic pH and naphthenic acids. Their results indicated that cattails exposed to oil sands effluents increased the photosynthetic rates, whereas cattails growing in non-impacted waters remained unchanged. Later, Kamaluddin and Zwiazek (2002) studied the effect of commercial naphthenic acids on aspen seedlings. They reported that naphthenic acids decreased root water flux, root respiration, leaf stomatal conductance, photosynthesis, chlorophyll concentration, and leaf size in the aspen seedlings. Subsequently, they measured the concentrations of naphthenic acids uptake by the roots in the xylem using Fourier transform infrared (FTIR) spectroscopy method and concentrations of 21 mg of naphthenic acids L^{-1} were reported. However, the FTIR method (Jivraj et al. 1995) is not specific for naphthenic acids because free fatty acids present in the xylem exudates would be also measured by this method. Thus, it is possible that the results of Kamaluddin and Zwiazek (2002) overestimate the concentrations of naphthenic acids uptake by aspen seedlings. Additionally, the uptake of organics compounds by plants is related to the logarithm of the compounds octanol/water partitioning coefficient, log K_{ow}. Compounds exhibiting lower hydrophobicity (log K_{ow} <1.8) will apparently not pass or pass more slowly through the lipid membranes of the root tissue, thus they do not accumulate at higher concentrations in the root tissue. In contrast with more hydrophobic compounds, which have good membrane permeability but lower xylem partitioning due to low water solubility, thus these chemicals may become bound to the root epidermis decreasing translocation (Burken and Schnoor 1998). Thus, complex mixture of compounds such as naphthenic acids whose log K_{ow} lies between 2 to 5 (Havre et al. 2003) may become available to roots of vascular plants such as cattails, but it is uncertain whether they are taken up by the xylem and translocated to its metabolic systems.

Recently, seepage from dike tailings has created contaminated adjacent wetlands where aquatic plants like cattails have been established as predominant vegetation (Bendell-Young et al. 2000). These grasses that grow in oil sands contaminated process-affected wetlands may accumulate naphthenic acids. As a result, in order to assess the uptake or accumulation of naphthenic acids in cattails a highly selective and sensitive analytical method is required. Thus, the objective of the present study was to develop a method to extract naphthenic acids from cattail leaves and to apply the gas chromatography-low resolution mass spectrometry (GC-LRMS) using reconstructed ion chromatograms (RICs, Murray et al. 2006) as described in Chapter 2 of this thesis to specifically detect naphthenic acids in the extracts for the nominal masses *m/z* = 265, 267 and 279. In the first part of this study, cattails leaves collected in areas not impacted with oil sands process-affected waters were spiked with West-In Pit (WIP) naphthenic acids in order to develop the method and to obtain a profile of fatty acids present in cattails. In the second part of the study, cattails plants collected in wetlands impacted with oil sands process-affected waters were collected and analyzed with the newly developed method, to test their applicability.

3.2 Materials and Methods

3.2.1 Naphthenic Acids Mixtures and Chemicals

In this study, only oil sands naphthenic acids were used. These acids were extracted from WIP waters from Syncrude Canada Ltd. as outlined in Section 2.2.1. All other chemicals and solvents were HPLC grade or analytical grade, and were purchased from Fisher or Aldrich-Sigma.

3.2.2 Plant Tissue Samples

Cattails (*Typha sp.)* were collected from the Beaumaris Lake at 153 Avenue and 109 Street in northwest Edmonton, Alberta and were used as off-site background plants for method development. Collected cattails were rinsed with tap water, their rhizomes were cut, and their leaves were folded and stored in plastic bags at -20°C until analysis.

3.2.3 Plant Tissue Spiked with WIP Naphthenic Acids

Cattails leaves were ground under liquid nitrogen to a fine powder with mortar and pestle (Conconi et al. 1996) and 5-g portions of the finely powered tissue was stored at -80°C in 60-mL vials. Five-gram of cattails leaves collected from Edmonton, Alberta were spiked with naphthenic acids at concentrations of 20 $\mu g\ g^{-1}$ and 5 $\mu g\ g^{-1}$ of plant tissues. Naphthenic acids were extracted from spiked samples with the procedure reported in Section 3.2.4. Additionally, 5 g of ground leaves that were not spiked with naphthenic acids were similarly extracted and analyzed by GC-LRMS to get a background fatty acids presents in plant tissues.

3.2.4 Extraction of Naphthenic acids from Plants Tissues

The extraction of naphthenic acids from plant tissues was performed according to Bligh and Dyer (1959) with the modifications outlined by Hamilton and Hamilton (1992). A 5-g sample of ground cattails leaves were transferred into a 50-mL flask containing 15 mL a solution of chloroform:methanol (2:1 $v\ v^{-1}$) and the tissue was homogenized using an Ultra Turrax (Model T18, IKA Works Inc., Wilmington, NC) for 3 min at 10,000 rpm. Then 5 mL of chloroform and 5 mL of 0.01 M HCl were added and the mixture was homogenized for further 30 s. After the homogenized sample was allowed to settle for a few minutes to complete separation of the two phases, the aqueous phase was removed with a 10-mL pipette and the organic phase was filtered thorough Whatman No. 1 filter paper with anhydrous Na_2SO_4 (3 g). Finally, the organic layer was concentrated in rotary evaporator, dried in a gentle stream of nitrogen and dissolved in 20 mL of hexanes.

3.2.5 Clean up of Naphthenic acids on Florisil Chromatography Column

The Florisil column chromatography method of Carroll (1961) was used to separate free fatty acids and naphthenic acids from the total plant lipids. The Florisil (12 g, 60-100 mesh; Fisher Scientific, Pittsburgh, PA, USA.) was poured into a column (2 x 30 cm) as slurry in 30 mL hexanes. The lipid extract dissolved in 20 mL of hexanes was loaded on the top of the Florisil column. Then, the Florisil column was washed twice with 40 mL each of hexanes at flow rate of 3 mL min^{-1} to eliminate some of the lipids and some of the pigments. Finally, free fatty acids and naphthenic acids were eluted from the column using 100 mL of 4% acetic acid in ether at the same flow rate.

3.2.6 Extraction of Naphthenic acids from the Ether Fraction

The plant extracts were a deep green color because of pigments that were co-extracted with the naphthenic acids, even after the lipid separation with a Florisil chromatographic column. Free fatty acids and naphthenic acids were separated from the ether fraction by extracting four times with 4 mL portions of an alkaline aqueous solution containing 4% of sodium carbonate (Kaniuga and Michalski 1978). The combined alkaline extracts were acidified with 12 M HCl to pH 2 and then were extracted three times with 5 mL of dichloromethane. The combine extracts were taken to dryness under a gently stream of nitrogen. Finally, the residue containing the free fatty acids and naphthenic acids were dissolved in fresh dichloromethane and transferred into a 2-mL vial for derivatization and for GC-MS analysis. The derivatization procedure was described in detailed in Chapter 2 Section 2.2.7.

3.2.7 Analyses of Plant Tissues by GC-LRMS

Typically, the extracts containing the derivatized free fatty acids and naphthenic acids were dissolved in 50 μL with dichloromethane and 2 μL of this solution were injected into a Varian Vista 6000 gas chromatograph connected to a VG 7070E magnetic sector mass spectrometer as described in Section 2.2.9.

3.3 Results

Approximately 5 g of cattails leaves finely powdered under liquid nitrogen with mortar and pestle were spiked with 20 μg WIP naphthenic acids g^{-1}. Then, the free fatty acids and naphthenic acids were extracted, cleaned up with a Florisil column, derivatized and analyzed by GC-LRMS. RICs for the nominal masses of 265, 267 and 279 that corresponds to naphthenic acids compounds that fit the formula $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$ and $C_{14}H_{22}O_2$ respectively, were acquired to determine whether the naphthenic acids could be detected among the background of lipids from the plant tissues. In addition, cattails leaves that were not spiked with naphthenic acids were also extracted and analyzed to provide "background" GC-MS data that were compared to the data from spiked samples.

One of the major problems working with plant tissues is the abundance of pigments that are co-extracted with the lipid fraction. Total concentration of chlorophyll in cattail was found to be 32.9 ± 8.2 mg mL^{-1} (Amaya-Chavez et al. 2006), while concentrations of naphthenic acids expected to detect in plants extracts are below 1 μg g^{-1}. Thus, in order to separate the pigment fraction from the lipids, the free fatty acids and naphthenic acids were extracted from the ether fraction with an alkaline aqueous solution containing 4% of sodium carbonate.

Cattail leaves that were spiked with 20 μg naphthenic acids g^{-1} showed a hump in the RICs for nominal *m/z* = 265, 267 and 279 between 15 to 23 min, indicating the presence of naphthenic acids (Figure 3.1B, C and D), whereas cattail leaves spiked with 5 μg naphthenic acids only showed a hump in the RIC for the nominal mass of 267 (Figure 3.2) with retention time between 15 to 20 min indicating that when lower concentrations of naphthenic acids are analyzed a greater sensitivity is required to detect the ions with the nominal mass of 265 and 279, which are less abundant in the mixtures of naphthenic acids than the ions with the nominal mass of 267 (See Table 2.1 for percentage of major ions in different naphthenic acids mixtures).

Additionally, cattails samples that were not spiked with naphthenic acids were extracted and analyzed in the same manner as spiked samples. RICs for the nominal masses of 265, 267 and 279 illustrated in Figure 3.3B, C and D did not showed any hump with retention time between 15 to 23 min as indicative of the presence of naphthenic acids, however, some peaks are observe in the retention time window of naphthenic acids, that correspond to fragments of fatty acids and did not interfere with the detection of naphthenic acids. The complexity of the plant tissues extracts can be seen in the total ion chromatogram (TIC) showed in Figure 3.1A and 3.3A.

3.4 Discussion

Preliminary results of the experiments with cattails leaves presented in this study (Section 3.3) exhibited poor sensitivity to detect naphthenic acids based on RICs chromatograms showed in Figure 3.1B, C and D for a spiked sample with 20 μg g^{-1}. This was likely due to the laborious extraction procedure combined with the abundance of pigments that were co-extracted with the solvents mixture. Because of time constraints, I was not able to continue developing the method for detecting naphthenic acids in plants. However Rozlyn Young, in our research group, continued developing this method (Appendix A).

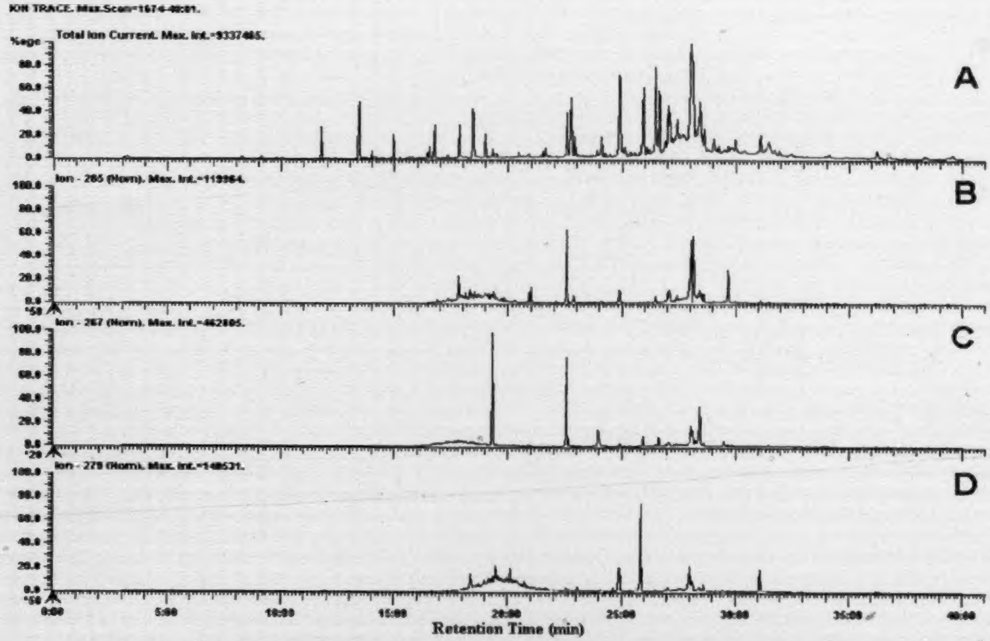


Figure 3.1 (A) TIC of the extract of 5 g of cattails leaves spiked with 20 μg g-1 of WIP naphthenic acids. RICs for the nominal mass of (B) *m/z*=265, (C) *m/z*= 267 and (D) *m/z*=279 showing a small hump characteristic of the presence of naphthenic acids.

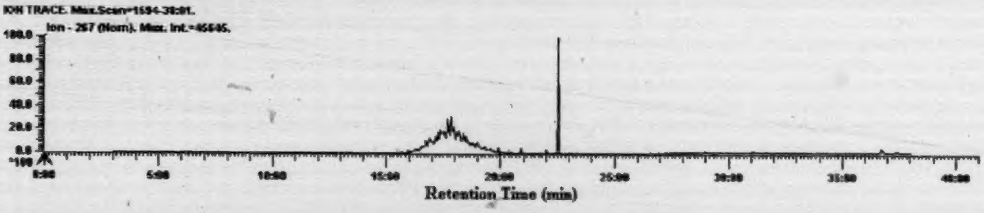


Figure 3.2 RIC for the nominal *m/z*= 267 of the extract of 5 g of cattails leaves spiked with 5 μg g^{-1} of WIP naphthenic acids that yield a small hump characteristic of the presence of naphthenic acids.

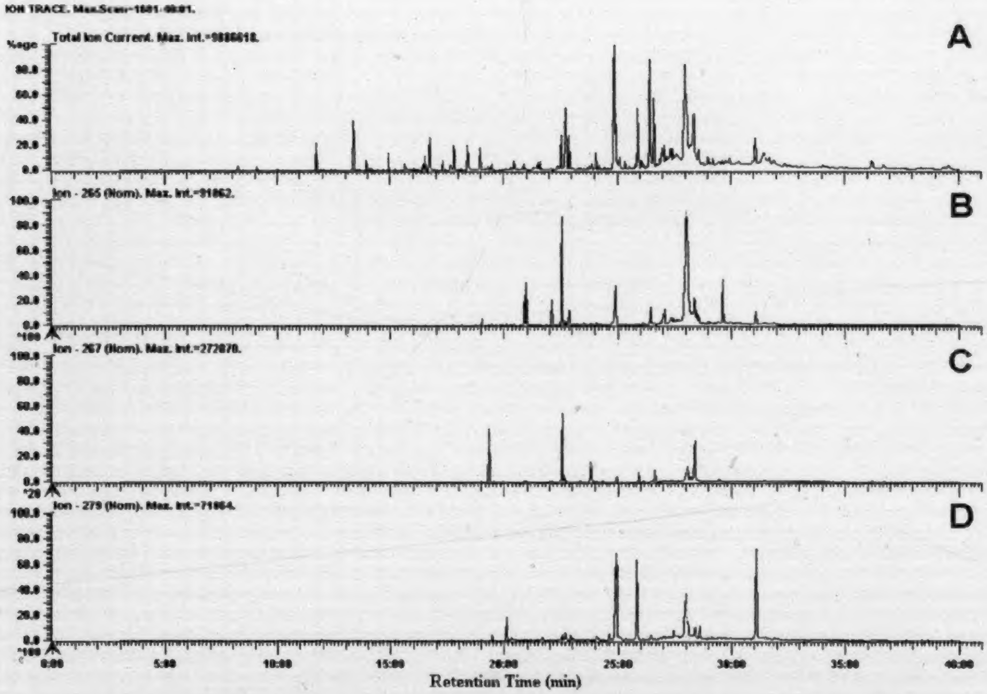


Figure 3.3 (A) TIC of the extract of 5 g of cattails leaves not spiked with WIP naphthenic acids. RICs for the nominal mass of (B) *m/z*=265, (C) *m/z*= 267 and (D) *m/z*=279 do not yield humps with retention time between 15 and 20 min.

Work by Young and Fedorak (Appendix A) describes an alternative clean up method based on the use of an anion exchange SPE column using formic acid and ether as eluent. Under these conditions, naphthenic acids were detected in spiked samples of cattails leaves at concentrations of 5 µg g^{-1}, but this concentration does not represent the instrumental detection of naphthenic acids in cattails and further studies are require to determine it.

In that procedure, the introduction of the SPE SAX column accomplish to separate the acid fraction containing the free fatty acids and naphthenic acids from the pigments and other compounds in one step, but the results from that study are only based on the detection of the ion with the nominal mass of 267 because this ion is typically the most abundant of the three ions selected and easily detectable at lower concentrations.

In addition, the work described in Appendix A attempted to detect the presence of naphthenic acids in cattails plants collected in oil sands ponds impacted with process-affected waters. Naphthenic acids were only detected in cattail roots and rhizome but not in the leaves. Even after soaking the roots in an aqueous solution of sodium carbonate at pH 12, the root and rhizome tissues showed the presence of naphthenic acids as well as the alkaline rinsing solution. These results validate the applicability of the method to detect naphthenic acids in cattails plants, but are limited to qualitative detection.

Using the non-specific FTIR method, Kamaluddin and Zwiazek (2002) reported naphthenic acids in the xylem of aspen seedlings after roots exposure. However, there is some question whether they were detecting naphthenic acids or naturally occurring fatty acids in the seedlings. The extraction, clean up and GC-MS methods described here and in Appendix A can provide the specificity needed for further studies addressing naphthenic acids uptake by plants.

3.5 Conclusions

The detection of naphthenic acids in plants tissues represents a challenge because the abundance of pigments and the high background noise present in the TIC that interfere with the analysis. In addition, concentrations lower than 5 $\mu g\ g^{-1}$ were not detected with the method presented in this chapter, however, the method described in Appendix A has the potential to detect lower concentrations. The use of the SPE SAX column removed the interferences caused by plant pigments, which were retained in the sorbent of the column resulting in cleaner chromatograms. Moreover, this method was applied to the detection of naphthenic acids in cattails exposed to oil sands effluents and the results confirmed the presence of these compounds on cattail roots and rhizomes. Further studies are required to improve selectivity and detection limit and to determine the extent to which naphthenic acids may be incorporated into the roots tissue or remain loosely associated with the roots.

3.6 Literature Cited

Amaya-Chavez, A., Martinez-Tabche, L., Lopez-Lopez, E. and Galar-Martinez, M. 2006. Methyl parathion toxicity to and removal efficiency by *Typha latifolia* in water and artificial sediments. Chemosphere, 63: 1124-1129.

Bendell-Young, L.I., Bennnett, K.E., Crowe, A., Kennedy, C.J., Kermode, A.R., Moore, M.M., Plant, A.L. and Wood, A. 2000. Ecological Characteristics of Wetlands Receiving and Industrial Effluent. Ecological Applications, 10: 310-322.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 910-917.

Burken, J.G. and Schnoor, J.L. 1998. Predictive relationships for uptake of organic contaminants by hybrid polar trees. Environmental Science and Technology, 32: 3379-3385.

Carrol, K.K. 1961. Separation of lipid classes by chromatography on florisil. Journal of Lipid Research, 2: 135-141.

Clemente, J.S. 2004. The characterization analyses and biodegradation of naphthenic acids. MSc. Thesis, University of Alberta, Edmonton, Canada

Clemente, J.S., Prasad, N.G.N., MacKinnon, M.D. and Fedorak, P.M. 2003. A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. Chemosphere, 50: 1265-1274.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology, 38: 1009-1016.

Conconi, A., Miguel, M., Browse, J.A. and Ryan, C.A. 1996. Intracellular levels of free linoenic and linoleic acids increase in tomato leaves in response to wounding. Plant Physiology, 111:797-803.

Crowe, A.U., Han, B., Kermode, A.R., Bendell-Young, L.I., and Plant, A.L. 2001. Effects of oil sands effluent on cattail and clover: photosynthesis and the level of stress proteins. Environmental Pollution, 113: 311-322.

Fan, T.-P. 1991. Characterization of naphthenic acids in petroleum by fast atom bombardment mass spectrometry. Energy and Fuels, 5: 371-375.

Hamilton, R.J. and Hamilton, S. 1992. Lipid analysis. A practical approach. IRL Press, Oxford. University Press, New York.

Havre, T.E., Sjoblom, J. and Vindstad, J.E. 2003. Oil/water-partitioning and interfacial behavior of naphthenic acids. Journal of Dispersion Science and Technology, 24: 789-801.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2001. Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research, 35: 2595-2606.

Jivraj, M.N., MacKinnon, M. and Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Analytical Methods Manual. Research Department, Syncrude Canada Ltd., Edmonton.

Kaniuga, Z. and Michalski, W.P. 1978. Photosynthetic apparatus of chilling sensitivity plants. II. Changes in free fatty acid composition and photoperoxidation in chloroplasts following cold storage and illumination of leaves in relation to Hill reaction activity. Planta, 140: 129-136.

Kamaluddin, M. and Zwiazek, J.J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. Tree Physiology, 22: 1265-1270.

Kasperski, K.L. 1992. A Review of properties and treatment of oil sands tailings. AOSTRA Journal of Research, 8: 11-52.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. 2003. Electrospray-mass spectrometric analysis of reference carboxylic acids and Athabasca oil sands naphthenic acids. Analytical Chemistry, 75: 6394-6400.

MacKinnon, M.D. 1989. Development of the tailings pond at Syncrude's oil sands plant: 1978-1987. AOSTRA Journal of Research, 5: 109-133.

MacKinnon, M.D., and Boerger, H. 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Canadian Journal of Water Pollution and Research, 21: 496-512.

Madill, R.E.A., Orzechowski, M., Chen, G., Brownlee, B.G. and Bunce, N.J. 2001. Preliminary risk assessment of the wet landscape options for reclamation of oil sands mine tailings: bioassays with mature fine tailings pore water. Environmental Toxicology, 16: 197-208.

Masliyah, J. 2002. Processing of tailings in Canadian oil sands industry. Transaction of Nonferrous Metals Society of China, 12: 524-528.

Murray, K.K., Boyde, R.K., Eberlin, M.N., Langley, G.J., Li, L. and Naito, Y. 2006. IUPAC Standard Definitions of Terms relating to Mass Spectrometry. IUPAC project 2003-056-2-500. http://www.msterms.com/docs/IUPAC_MS_Terms_Second_Draft.pdf

Roques, D.E., Overton, E.B. and Henry, C.B. 1994. Using gas chromatography/mass spectrometry fingerprint analysis to document process and progress of oil degradation. Journal of Environmental Quality, 23: 851-855.

Seifert, W.K., Teeter, R.M., Howells, W.G. and Cantow, M.J.R. 1969. Analysis of crude oil carboxylic acids after conversion to their corresponding hydrocarbons. Analytical Chemistry, 41: 1638-1647.

Tomczyk, N.A., Winans, R.E., Shinn, J.H. and Robinson, R.C. 2001. On the nature and origin of acidic species in petroleum. 1. Detailed acid type distribution in a California crude oil. Energy and Fuels, 15: 1498-1504.

Yen, T.-W., Marsh, W.P., MacKinnon, M.D. and Fedorak, P.M. 2004. Measuring naphthenic acids concentrations in aqueous environmental samples by liquid chromatography. Journal of Chromatography A, 1033: 83-90.

4. Detection of Naphthenic Acids in Rat Plasma and Rat Liver by Gas Chromatographic - Mass Spectrometry

4.1 Introduction

Naphthenic acids are a complex mixture of acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number and Z specifies the homologous families ranging from 0 to -12 (Clemente et al. 2004). Acyclic compounds with Z=0 are highly branched rather than being simple linear fatty acids, which also fit the formula for naphthenic acids with Z=0 (Rudzinksi et al. 2002). Cyclic compounds with Z=-2 are single-ring structures with a 5- or 6-membered ring. As Z decreases from Z=-4 to Z=-12 the cyclic structures became more complex with different combinations of fused or bridged rings (Brient et al. 1995).

Naphthenic acids are constituents of conventional crude oils in small quantities (Slavcheva et al. 1999), and of the Athabasca oil sands ores (Clemente et al. 2004) near Fort McMurray, Alberta, Canada. These acids originate from the partial biodegradation of petroleum by native microorganisms (Tissot and Welte 1978). The oil sands industry separates bitumen from sand by the Clark hot water extraction process, in which the mined ore is mixed with hot water, sodium hydroxide and air, so the bitumen floats to the surface as froth, which is collected and upgraded to a synthetic crude oil (Schramm et al. 2000). After the bitumen is removed, the slurry of water containing sand, clay, residual bitumen and other inorganic and organic compounds is pumped into artificial settling ponds (MacKinnon 1989, Kasperski 1992, MacKinnon and Boerger 1986). During the separation of the bitumen, the alkaline pH of the water (between 8 and 9) dissolves naphthenic acids as sodium naphthenates that concentrate in the tailings waters. Tailings pond waters contain naphthenic acids in concentrations that vary from 20 to 120 mg L^{-1} (Holowenko et al. 2002). Because naphthenic acids are toxic to aquatic species, tailings waters cannot be released into the environment (CONRAD 1998, Lo et al. 2003,).

Aquatic toxicity studies have exposed fish to naphthenic acids or tailings waters containing naphthenic acids (Dokholyan and Megomedov 1983, MacKinnon and Boerger 1986, Nix and Martin 1992, Verbeek et al. 1994, van den Heuvel et al. 1999a, 1999b, 2000, Nero et al. 2006). The results from these studies indicated that naphthenic acids are toxic to fish and induce numerous effects at different levels like changes in the biochemical profile, tissues damage and necrosis, or deformities.

In addition, plants (Kamaluddin and Zwiazek 2002) and animals (Rogers et al. 2002a) have shown adverse effects when they were exposed to naphthenic acids. For example, Rogers et al. (2002a) reported a mammal toxicity study using Wistar rats as a model. The rats were exposed to naphthenic acids from commercial and oil sands sources in acute and chronic treatments; the results showed severe damage to the liver and necrosis to other tissues. In a plant toxicity study, Kamaluddin and Zwiazek (2002) exposed aspen seedlings to different concentrations of commercial naphthenic acids and observed decreased growth and photosynthetic rates. They measured the amount of naphthenic acids uptake by aspen seedlings using Fourier transform infrared (FTIR) spectroscopy method. However, the FTIR method (Jivraj et al. 1995) lacks specificity to detect naphthenic acids only, because naturally occurring carboxylic acids, which are abundant in biological tissues, are also detected by this method. In Section 2.2.9 of this thesis, a method to specifically detect naphthenic acids in water samples by gas chromatography-low resolution mass spectrometry (GC-LRMS) using reconstructed ion chromatograms (RICs, Murray et al. 2006) for nominal mass *m/z* = 267 was described. Recently, Young et al. (2007) applied the GC-MS method outlined in Chapter 2 to detect naphthenic acids in fish that were exposed to oil sands tailings water or fed with commercial naphthenic acids. Their results showed that naphthenic acids can be selectively detected in fish.

In this work, an analytical method to extract naphthenic acids from rat liver tissue and plasma, and the use of the GC-MS method to specifically detect naphthenic acids in liver and plasma extracts are described. In the first part of the study, liver samples from healthy rats were spiked with Merichem naphthenic acids in order to develop the method and to obtain background information on of the fatty acids present in the tissue extracts. In the second part of the study, ten Wistar rats were orally fed with a solution of Merichem naphthenic acids to a concentration of 300 mg kg^{-1} by weight and six Wistar rats were fed with tap water and used as reference. Small amounts of naphthenic acids were detected in the liver of rats fed with Merichem naphthenic acids base on RICs for nominal mass *m/z* = 267. Plasma samples from rats fed with naphthenic acids were also analyzed by GC-MS, but naphthenic acids were detected in only one of four samples tested.

4.2 Material and Methods

4.2.1 Naphthenic Acids Mixtures and Chemicals

The commercial preparation of naphthenic acids used in this study was refined Merichem acids obtained as a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX). A stock solution containing 1 mg of naphthenic acids mL^{-1} was further diluted to working concentrations (e.g., 100 μg naphthenic acids mL^{-1}). These solutions were stored in vials with Teflon-lined cap at -20°C. The derivatizing reagent *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluorocetamide (MTBSTFA) that contains 1% *tert*-butyldimethylsilylchloride was purchase from Sigma (Sigma, St. Louis, MO). The remaining organic solvents used for the extraction and analyses of naphthenic acids were HPLC-grade or ACS certified and were purchased from Fisher or Caledon.

4.2.2 Biological Samples

Biological samples, including livers and plasma from Sprague-Dawley rats that had not been exposed to naphthenic acids were used for method development. These materials were obtained as a gift from Anooshiruan Shayeganpour from the Faculty of Pharmacy or were purchased in the Biosciences Animal Services at the University of Alberta. Frozen horse serum used for the radioactivity experiments was purchased from Cansera (Etobicoke, Ontario). All biological samples were stored at -20°C in individual containers and were thawed at room temperature prior to the extraction.

4.2.3 Rat Plasma Spiked with Merichem Naphthenic Acids

Samples of rat plasma (1 mL) were spiked with different amounts of Merichem naphthenic acids (1 μg or 0.5 μg) to assess the detection limit of this method. The spiked samples were extracted according to the method of Mehta et al. (1998) as outlined in Section 4.2.6. Rat plasma samples (1 mL) that were not spiked with naphthenic acids served as control samples.

4.2.4 Rat Liver Samples Spiked with Merichem Naphthenic Acids

Five-gram samples of rat liver were spiked with Merichem naphthenic acids to final concentrations of 1 $\mu g\ g^{-1}$ or 0.5 $\mu g\ g^{-1}$ in order to establish the minimum detectable concentrations of this method. The spiked livers were extracted according to the method reported by Young et al. (2007) outlined in Section 4.2.7. In addition, 5 g of rat liver samples not spiked with naphthenic acids were used as control samples.

4.2.5 Rats Exposed to Merichem Naphthenic Acids

Sixteen healthy Wistar rats were used for the naphthenic acids exposure experiments done at the Toxicology Centre University of Saskatchewan. Treated animals were dosed by gavage using a stainless steel feeding needles. All animal procedures were approved by the University of Saskatchewan Committee on Animal Care and Supply in accordance with the principles of Animal Care of the Canadian Federation of Biological Societies. The rats were divided in two groups. Group A was a control group composed of six rats identified with numbers from 1 to 6. Group A was dosed orally with a feeding needle with tap water at 5.5 mL per kg of rat weight. Group B comprised ten rats that were identified with numbers 7 to 16. Group B was administered with a single oral dose of an aqueous solution of Merichem naphthenic acids at 5.5 mL kg^{-1} of rat weight to a final concentration of 300 mg of naphthenic acids kg^{-1} body weight. After 6 h, the rats from both groups were anesthetized by inhalation with isoflurane, and approximately 4 mL of blood was collected into heparinized tubes from each rat. Half of the blood samples (rats 1 to 3 and 7 to 11) were transferred to cryotubes and frozen at -20°C as whole blood (two vials per rat containing approximately 1.8 mL each), the other half of the blood samples (rats 4 to 6 and 12 to 16) were centrifuged, and the plasma – the liquid portion of non-clotted blood that contains the coagulation factors (Luque-Garcia et al. 2006) – was transferred to 2-mL cryotubes (one vial per rat containing approximately 1.5 mL of plasma each) and frozen at -20°C. Then, the livers were removed and each liver was placed in a separated sterile plastic bag (Whirl Pac) and stored at -20°C until further analysis.

The dose delivered and dosing period of Merichem naphthenic acids were selected according to the earlier study conducted by Rogers et al. (2002a), who established that the dose of 300 mg per kg body weight reflects 50 times the worst-case exposure to naphthenic acids in a single day exposure for wild animals.

4.2.6 Extraction of Naphthenic Acids from Rat Plasma

Free fatty acids and naphthenic acids were extracted from plasma using the method of Mehta et al. (1998) with some modifications. Specifically, a 1000 μL sample of heparinized plasma was extracted with 5 mL of a solution containing 2-propanol: heptane: 6 M HCl (40:10:1 by volume) in a 15-mL test tube with a Teflon lined screw cap. The sample was vigorously mixed using a vortex (Genie 2, Fisher Scientific, Ontario, CA) at speed 5 for 30 s. Then, the samples were immersed in a water bath sonicator (Ultrasonic cleaner model FS30, 130W, Fisher Scientific) at room temperature for 2 min at intervals of 30 s on and 30 s off. Following that, the samples were again vigorously mixed for 1 min in a vortex at speed 10 and allowed to sit at room temperature for 10 min to separate the liquid layers. Then, 2 mL of heptane and 3 mL of 0.01 M HCl were added to each sample and the tubes were thoroughly vortexed at speed 10 and sonicated for 1 min. Finally, the tubes were centrifuged at speed 5 in a bench-top centrifuge (International Clinical Centrifuge, Model CL) for 10 min at 4°C.

A 3-mL portion of the upper (heptane) layer was separated from the plasma and evaporated to dryness under a stream of nitrogen. The residue, containing the extracted carboxylic acids was cleaned up with a solid-phase extraction column as described in Section 2.2.6. The eluted acids were dried under nitrogen, dissolved in 50 µL of dichloromethane and then converted to their *tert*-butyldimethylsilyl derivatives as outlined in Section 2.2.8. Typically, the derivatized residue was dissolved with 25 µL of dichloromethane and 2 or 3 µL of this mixture were analyzed by GC-MS.

To estimate the recovery of naphthenic acids and free fatty from spiked serum, 140,000 dpm of [1-^{14}C] stearic acid (New England Nuclear, Boston, MA) were added to a 500-µL sample of horse serum and this was extracted as outlined in Section 2.2.6 Aliquots of the heptane layer that contained the free fatty acids were added to 10 mL ACS fluor (Aqueous Counting Scintillant, Amersham Canada Ltd, Oakville, Ontario) and the amount of recovered radioactivity was measured using ad Beckman model LS 3801 liquid scintillation counter. After scintillation counting, the efficiency of the extraction method was calculated.

4.2.7 Extraction of Naphthenic Acids from Rat Liver

Free fatty acids and naphthenic acids from rat liver samples were extracted using the method reported by Young et al. (2007). All glassware used in this procedure was thoroughly cleaned and rinsed with dichloromethane prior to use. Approximately 5 g of rat liver tissue was placed in a 250-mL Erlenmeyer flask, then 100 mL of chloroform: methanol 2:1(by volume) was added to the mixture and this was homogenized for 2 min using an Ultra Turrax (Model T18 basic, IKA Works Inc., Wilmington, NC) homogenizer at 10,000 rpm. After 2 min, 25 mL of a solution of 0.11 M NaCl acidified to pH 2 with 6 M or 12 M HCl were added to the mixture and homogenized for 1 min. Then the homogenized tissue was allowed to sit for about 20 min or until the liquid layers separated. Next, the chloroform phase, containing the lipid fraction and the naphthenic acids was removed from the bottom of the flask using a 10-mL glass pipette and 50 mL of chloroform was added to the homogenate. The mixture was homogenized for 30 s, and then the chloroform layer was removed, and another 50 mL of chloroform were added, homogenized and removed as previous described above. The pooled chloroform layers, containing the free fatty acids and naphthenic acids, were extracted with three portions of 25 mL of an aqueous solution containing 4% (wt v^{-1}) sodium carbonate (pH 11.6). The alkaline aqueous extracts were combined and acidified with 12 M HCl to pH 2 and extracted with three 20-mL portions of dichloromethane. The dichloromethane extracts were combined and the solvent was evaporated under a flow of nitrogen and the residual carboxylic acids were dissolved in 1 mL of hexanes.

Naphthenic acids and free carboxylic acids were isolated from the total lipids using a solid-phase extraction column with strong anionic exchanger (SAX). This procedure was adapted from Jones et al. (2001) and is outlined in Section 2.2.6.

Purified naphthenic acids and free fatty acids residues were dissolved in 500 μL of dichloromethane and this solution was mixed with 500 μL of *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluorocetamide to derivatize the carboxylic acids. This mixture was allowed to react in a water bath at 60°C for 20 min. Finally, the reaction mixture was evaporated to dryness and the residue was dissolved in 50 µL of dichloromethane. Typically, 2 µL of the *tert*-butyldimethylsilyl derivatives were analyzed by GC-MS.

4.2.8 Analysis of Naphthenic Acids by GC-Low Resolution MS (GC-LRMS)

Samples of derivatized residues were injected into the GC-MS described in Section 2.2.9. Data acquisition was carried out in full mass range scanning from *m/z* 550 to 150. The total ion chromatograms (TIC) and the RICs for an ion with a nominal mass *m/z*=267 were acquired.

To improve the detection of naphthenic acids by GC-LRMS, the conditions in the MS were changed to acquire the ion current within a selected narrow mass range *m/z* 264.5 to 270 that included the characteristic ions of the naphthenic acids with the nominal mass of 267 in the expected retention time window rather than scanning the entire mass range *m/z* 150 to 550. In narrow mass range scanning, the mass spectrometer does not spend time scanning the full mass range, but rapidly changes between *m/z* values from which characteristic ions are expected. This procedure allowed measurements within a limited number of masses with high sensitivity and high specificity, however, are often accompanied by an increase in the signal-to-noise ratio due to column bleed or matrix interferences (Lawson 1989) The narrow mass range scanning produced a TIC from which the RIC for *m/z* = 267 is extracted while the conditions in the GC remain as outlined in Section 2.2.9. Operating conditions of the MS for narrow scan mode are reported in Appendix B.

4.2.9 Analysis of Naphthenic Acids by GC-High Resolution MS (GC-HRMS)

GC-HRMS analyses of *tert*-butyldimethylsilyl derivatives of naphthenic acids and free fatty acids were performed using a Kratos MS50 mass spectrometer using the same settings for single ion monitoring (SIM) as reported in Section 2.2.10.

4.3 Results and Discussion

A GC-MS method to specifically detect naphthenic acids in water using RICs from the GC-MS analyses of derivatized extracts was described in Chapter 2. This method monitored three specific ions with nominal masses *m/z* of 265, 267 and 279, which corresponds to the base fragments of *tert*-butyldimethylsilyl derivatives of naphthenic acids with the general formulas of $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$ and $C_{14}H_{22}O_2$ respectively. Because each formula represents dozens of isomers that cannot be resolved in the chromatographic column, the RIC of these three major ions yield humps with retention times between 15 and 23 min. Thus, the appearance of a hump in the RIC is indicative of the presence of naphthenic acids in the samples. Similarly, Young et al. (2007) use the same approach as outlined in Chapter 2 to detect naphthenic acids in fish tissues. However, they only monitored the ion with nominal mass of 267 because this ion is the most abundant of the three ions selected to detect naphthenic acids and was easily detected in fish tissues at concentrations of 1 $\mu g\ g^{-1}$. Furthermore, naturally occurring fatty acids with 13 carbons and two unsaturations were not found in fish tissues (Morris et al. 2005, Young et al. 2007). Therefore, RICs for a nominal mass of 267 with retention times between 15 to 20 min are very specific to detect naphthenic acids. Fatty acids such as hexadecanoic acid, octadecanoic acids and 9,12-octadecadienoic acid were also present in fish tissue extracts (Young et al. 2007) and were detected in the chromatograms with naphthenic acids. 9,12-Octadecadienoic acid also produces a fragment ion with nominal mass of 267 which appeared in the RIC at a later retention time than the *m/z* = 267 hump from the naphthenic acids ions (15 to 20 min). Although the *m/z* = 267 ions from the 9,12-octadecadienoic acid did not co-elute with those of the naphthenic acids, these ions from 9,12-octadecadienoic were very abundant and they saturated the response of the MS that occurs when the integrating system disregard that part of the signal that exceeds 10 V. To eliminate the chance of saturating the response of the MS, the mass of the sample introduced into the GC-MS had to be reduced by injecting a smaller volume of sample, or by diluting the sample prior to injection.

Although Scott et al. (2005) demonstrated that naphthenic acids from commercial mixtures and oil sands tailings waters have different acid composition, the results from fish tissues (Young et al. 2007) and water samples (Chapter 2) provide further evidence that the sensitivity of the GC-LRMS method was similar in both studies when the two sources of naphthenic acids was tested. Thus, in the present study only Merichem naphthenic acids were used through all the experiments.

4.3.1 Detection of Naphthenic Acids in Rat Plasma Spiked with Merichem Acids

During the method development, heparinized plasma samples from healthy rats were used and spiked with 1 μg or 0.5 μg of Merichem acids mL^{-1}. Initially, free fatty acids and naphthenic acids were extracted according to the method of Mehta et al. (1998), which used 2 M phosphoric acid in the extraction mixture. However, the results from the GC-LRMS analysis showed that traces of this acid in the extracts were derivatized to phosphoric acid tris(*tert*-butyldimethylsilyl) ester, as identified by the NIST computer library matching program in the GC-MS. A derivatized phosphoric acid produced a fragment ion with nominal mass of 267 that eluted as part of naphthenic acids hump as illustrated in Figure 4.1A, 4.1B and Figure 4.2A. Consequently, to avoid the interferences caused by phosphoric acid in the GC-MS analysis, 6 M HCl was used in place of phosphoric acid and no interference was noted in the RIC as shown in Figure 4.1C. The total ion chromatogram (TIC) of a spiked plasma sample analyzed by GC-MS is illustrated in Figure 4.1A, showing the profile of fatty acids that are co-extracted with naphthenic acids.

To assess the extraction efficiency of this method, four samples of horse serum were spiked with [1-^{14}C] stearic acid and the amount of the radioactivity recovered in the heptane extract was measured using a Scintillation counter. Based on that measurements the average extraction recovered was 80 ± 8% of the [1-^{14}C] stearic acid. In addition, to assess the detection limit of this method, plasma samples spiked with 0.5 μg or 1 μg of Merichem naphthenic acids mL^{-1} were extracted (with the modifications reported in Section 4.2.6), cleaned up with a SAX column, derivatized and analyzed by GC-LRMS in full mass range mode. The RICs for nominal *m/z* value of 267 from those samples are shown in Figures 4.1B and 4.2B, where a small hump with retention time between 15 to 20 min was present in the RIC of both chromatograms indicating the presence of naphthenic acids. Plasma samples spiked with 1 μg mL^{-1} gave the same results when duplicate samples were analyzed, whereas plasma samples spiked with 0.5 μg of naphthenic acids mL^{-1} required amplification of the response of the MS or repeat the extraction in order to obtain the same results in duplicated samples. Thus, the lower detection limit for this method is about 1 μg mL^{-1}. No naphthenic acids were detected in the RIC of an extract of 1 mL of plasma sample that was not spiked with naphthenic acids (Figure 4.3). Some peaks were observed in the retention time window of naphthenic acids in not spiked samples, however, these peaks did not produce a hump or interfere with the detection of naphthenic acids in spiked samples.

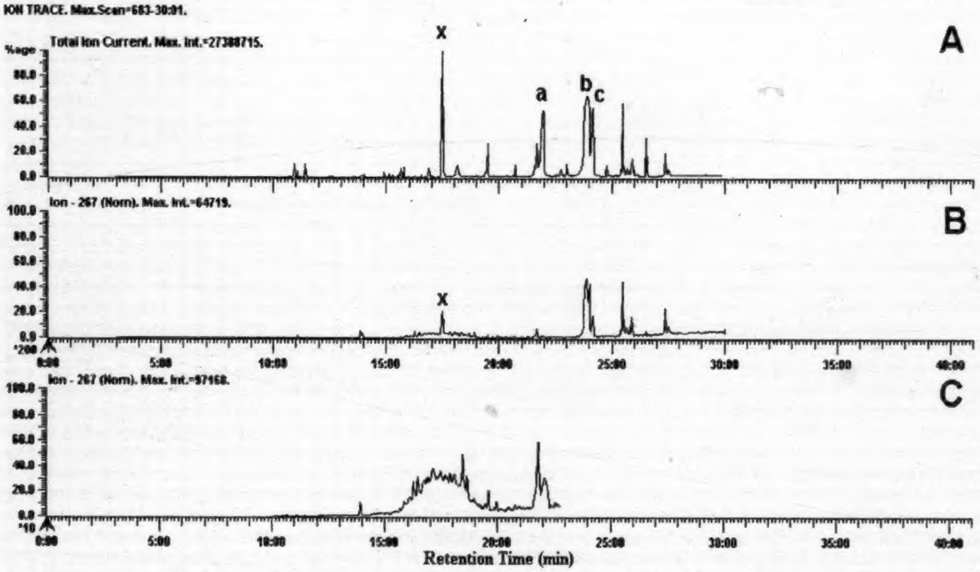


Figure 4.1 (A) TIC from the extract of 1 mL of rat plasma spiked with 1 μg mL^{-1} of Merichem naphthenic acids; (B) RIC for *m/z* =267 that yield a hump with retention time between 15 and 20 min indicating the presence of naphthenic acids in full mass range scan mode and (C) RIC for *m/z* =267 in narrow mass range scan mode. Peak (x) is from derivatized phosphoric acid used during the initial extraction procedure (A and B) and did not appear when phosphoric acid was replaced with HCl (C) in the extraction method. Resolved peaks of derivatized fatty acids can be observed in the TIC where the most abundant are the *tert*-butyldimethylsilyl esters of hexadecanoic acid (a), 9,12-octadecadienoic acid (b) and octadecanoic acid (c).

To improve the sensitivity of this method, 1-mL plasma samples spiked with 0.5 μg or 1 μg of Merichem naphthenic acids were analyzed by GC-LRMS in narrow mass range scan mode. An example of the detection of naphthenic acids using narrow mass range scanning in rat plasma spiked with 1 μg mL^{-1} is illustrated in Figure 4.1C, where a hump characteristic of the presence of naphthenic acids is observed in the RIC chromatogram. In this mode, the MS monitored only a few masses around the nominal mass of 267 at slower scanning rate than in full mass range scan mode, thus each mass is measured more accurately at a very high sensitivity. Unfortunately, this increase in sensitivity also translated into an increased signal-to-noise due to column bleed or matrix interferences that raised the baseline after 21 min retention time, but this baseline rise did not affect the detection of naphthenic acids hump in the RIC for the nominal mass of 267 which eluted earlier (from 15 to 20 min). Because the instrument used for the GC-LRMS analysis lacks the resolving power to analyze single ions, a range of masses between 270 to 264.5 was monitored instead and the RIC acquired for nominal $m/z = 267$ represents a range between 267.0 to 267.7.

A major problem to overcome in trace analysis of naphthenic acids extracted from biological tissues and analyzed in full mass range scan mode is the presence of interfering masses from fatty acids, which have higher relative intensity than naphthenic acids 267 ions, so the hump from naphthenic acids at low concentrations can only be distinguished when the chromatogram is enlarged. Narrow mass range mode reduced the effects of interferences from co-eluting compounds because only ions within the range of masses (*m/z* 270 to 264) are monitored during the GC-elution in a close retention time. In this mode, the MS remained off during the first 10 min of the run, and started acquiring data only during the retention time from 10 to 23 min. After 23 min, the MS was turned off again whereas the temperature program of the GC continued to a total run time of 40 min.

Sample extracts containing 1 μg or 0.5 μg of Merichem naphthenic acids mL^{-1} were dissolved in 20 or 25 μL of dichloromethane and 2 to 3 μL of the extract were injected into the GC-LRMS and analyzed by either full mass range scan or narrow mass range scan, the results from those analyses showed that narrow mass range scan improve the results as concentrations as low as 0.5 μg mL^{-1} (Figure 4.2B) could be detected. In contrast, when an extract of 0.5 μg of naphthenic acids mL^{-1} was analyzed in full mass range the characteristic hump that appeared between 15 to 20 min was hardly seen at 100% of the relative intensity of the chromatogram as shown in Figure 4.2A. Based on these results, the detection limit of this method is about 0.5 μg mL^{-1} when narrow mass scan is used and no naphthenic acids were detected in the RIC chromatograms of plasma samples with no naphthenic acids added as shown in Figure 4.3. Thus, the plasma samples from rats fed with Merichem naphthenic acids were analyzed in the narrow scan mode.

4.3.2 Detection of Naphthenic Acids in Rat Plasma from Rats fed with Merichem Acids

This study was conducted to determine if naphthenic acids could be detected in plasma extracts from rats fed with naphthenic acids and sacrificed 6 h after ingestion. Four plasma samples from rats fed 300 mg of Merichem naphthenic acids kg^{-1} of body weight were extracted, cleaned up, derivatized and analyzed by GC-LRMS in similar manner as spiked plasma samples. Naphthenic acids were not detected in three of the plasma samples, from the exposed group B, based on RIC chromatograms. An example is provided in Figure 4.4B. However, a small hump in the RIC with retention time between

15 to 20 min, corresponding to naphthenic acids, was detected in plasma extracts from naphthenic acids-fed rat #12 (Figure 4.4A). GC-LRMS results from the plasma extract #12 demonstrate the ability of this method to detect naphthenic acids in plasma samples. However, this method is strictly qualitative, where a sample is assigned as positive when a hump is detected in the RCI for *m/z* = 267 with retention time between 15 to 20 min, no concentrations of naphthenic acids can be reported.

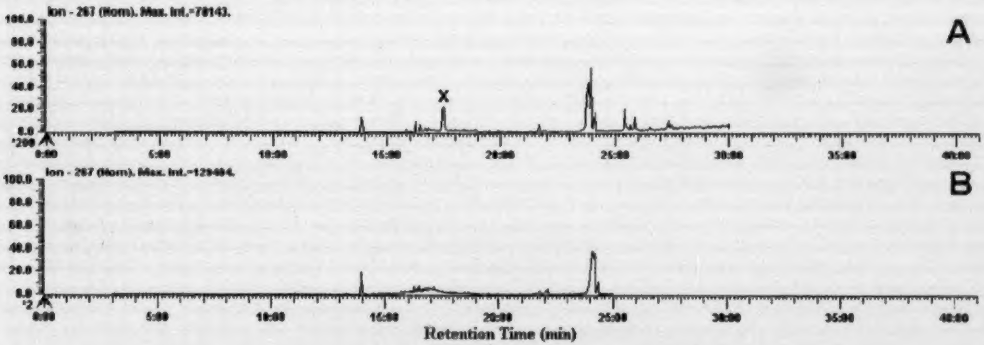


Figure 4.2 Analysis of the extract of 1 mL of rat plasma spiked with 0.5 μg mL^{-1} of Merichem naphthenic acids, where (A) is the RIC for the *m/z* =267 in full mass range scan mode and (B) is the RIC for the *m/z*=267 acquired in narrow mass range scan mode, when phosphoric acid was replaced with HCl, that yield a hump with retention time between 15 and 20 min indicating the presence of naphthenic acids. Peak (x) is from derivatized phosphoric acid used during the initial extraction procedure.

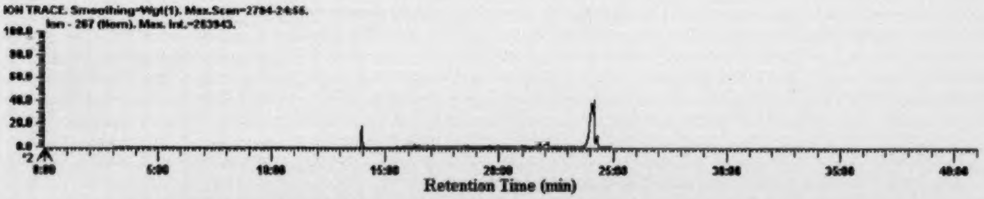


Figure 4.3 Analysis of the extract of 1 mL of rat plasma sample not spiked with naphthenic acids and acidified with HCl. RIC for the nominal mass of *m/z* =267 in narrow mass range scan do not yield a hump that indicated the presence of naphthenic acids.

In addition, four-control plasma samples from rats fed with tap water were analyzed by GC-LRMS. Naphthenic acids were not detected in any of the control samples analyzed by GC-MS based on RIC chromatograms. However, some peaks are shown in the region between 15 to 20 min as illustrated in Figure 4.5 that corresponds to fragment ions from fatty acids and other unidentified compounds.

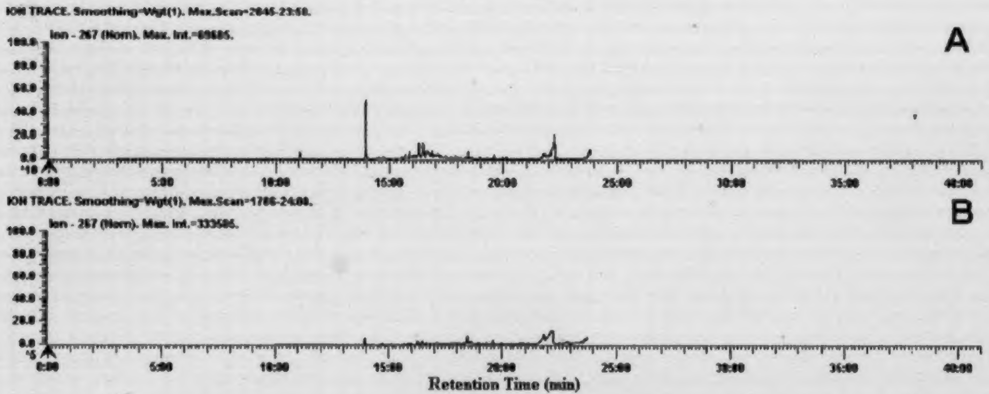


Figure 4.4 (A) RIC for *m/z*=267 that yielded a small hump with retention time between 15 to 20 min indicating the presence of naphthenic acids in 1 mL of plasma sample from naphthenic acids-fed rat #12 and (B) RIC for *m/z*=267 with no hump in the retention time between 15 to 20 min from naphthenic acids-fed rat #13. Both chromatograms were acquired in narrow mass range scan.

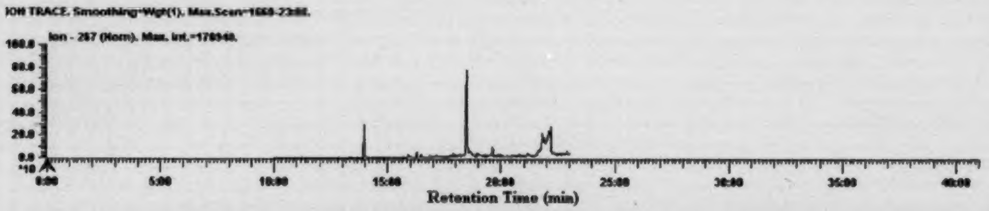


Figure 4.5 Analysis of the extract of 1 mL of plasma from rat #5 from the control group A that was not fed with naphthenic acids. The RIC for *m/z*=267 was acquired in narrow mass range scan mode with no hump in the retention time window of naphthenic acids.

4.3.3 Detection of Naphthenic Acids in Rat Liver Spiked with Merichem Acids

Five-gram liver samples from healthy Sprague-Dawley rats were spiked with 1 μg or 0.5 μg of Merichem naphthenic acids g^{-1} of tissue (equivalent to 1 mg kg^{-1} or 0.5 mg kg^{-1}) in order to develop the method and to establish the minimum amount detectable. After the naphthenic acids were extracted from the spiked rat tissue, the extracts were cleaned up with a SAX column and the purified acid fraction derivatized and analyzed by GC-LRMS using full mass range scan mode. Figures 4.6 and 4.7 show the GC-MS results from the analyses of the naphthenic acids extracted from these spiked samples. The TIC (Figures 4.6A and 4.7A) show typical peaks of fatty acids such as hexadecanoic acid, octadecanoic acid and 9,12-octadecadienoic acid that appear in the chromatograms as reported by Young et al. (2007). RICs from each of the spiked samples gave a hump that is characteristic of naphthenic acids (Figures 4.6B and 4.7B). In addition, rat liver samples that were not spiked with naphthenic acids were extracted and analyzed in the same manner. The results from the GC-MS analysis of one of these samples are shown in Figure 4.8. The RIC for nominal *m/z* values of 267 yielded no humps between 15 to 20 min (Figure 4.8B), but showed the presence of a few resolved peaks that correspond to compounds with fragment ions of *m/z*=267, and numerous ions with *m/z* 267 eluting between 25 to 26 min. These ions do not produce humps such as the ones observed in Figures 4.6B or 4.7B. Thus, there is no indication of any material in the unspiked liver extracts that resembles naphthenic acids in the profile of fatty acids extracted from rat liver tissues.

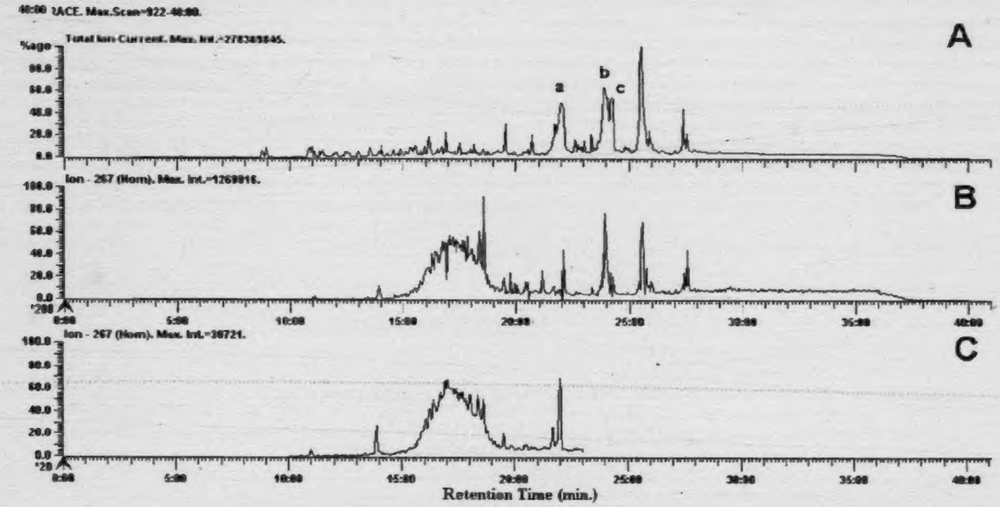


Figure 4.6 (A) TIC of the extract of 5 g of rat liver tissue spiked with 1 µg of Merichem naphthenic acids g^{-1}. (B) RIC for the nominal mass of *m/z*=267 that yielded a hump with retention time between 15 and 20 min characteristic of the presence of naphthenic acids acquired in full mass range scan mode and (C) in narrow mass range scan mode. See Figure 4.1 for identities of a, b and c.

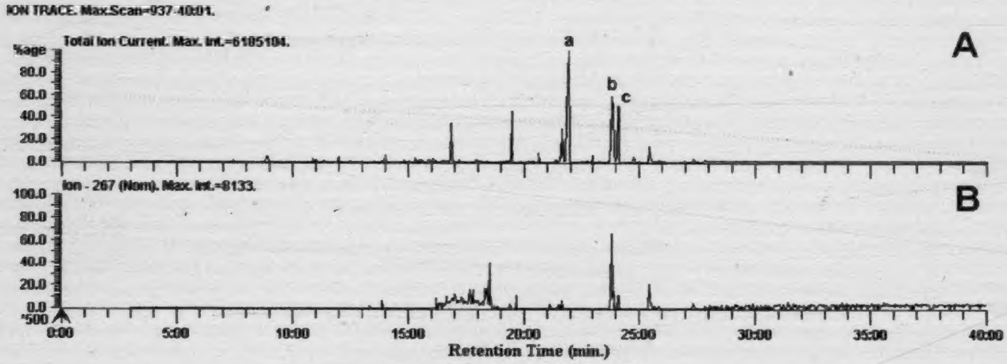


Figure 4.7 (A) TIC of the extract of 5 g of rat liver tissue spiked with 0.5 µg of Merichem naphthenic acids g^{-1}. (B) RIC for the nominal *m/z*=267 yielded a small hump with retention time between 15 to 20 min, acquired in full mass range scan mode. See Figure 4.1 for identities of a, b and c.

Similar to plasma extracts, liver extracts spiked with 1 μg or 0.5 μg of naphthenic acids were analyzed by GC-LRMS in narrow mass range scan mode in order to improve the detection limit of this method, and the results from the RIC (*m/z* =267) are shown in Figure 4.6C. Again narrow mass range scan mode improved the detection of naphthenic acids in liver samples as the naphthenic acids hump was easily discernable at 100% of the relative intensity in the chromatogram (Figure 4.6C). Based on these results, the detection limit of this method was established to be approximately 0.5 μg of naphthenic acids g^{-1} of tissue.

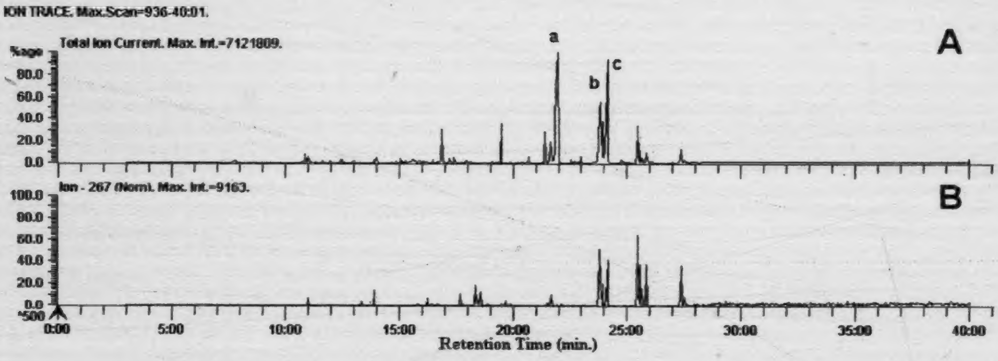


Figure 4.8 (A) TIC of the extract of 5 g of rat liver tissue not spiked with naphthenic acids. (B) RIC for the nominal *m/z*=267, which does not yield any hump with retention time between 15 to 20 min, acquired in full mass range scan. See Figure 4.1 for identities of a, b and c.

4.3.4 Detection of Naphthenic Acids in Rat Liver samples from Rats Fed with Merichem Acids

Four liver samples from rats fed with 300 mg of Merichem naphthenic acids kg^{-1} by weight were similarly extracted, cleaned up with a SAX column, derivatized and analyzed by GC-MS similar to the spiked liver samples. The TIC (Figure 4.9A) was nearly identical to the extract of liver samples spiked with naphthenic acids (Figure 4.6A) showing the *tert*-butyldimethylsilyl derivatives of fatty acids hexadecanoic acid, octadecanoic acid and 9,12-octadecadienoic acid. The results from the GC-MS analyses in all four of the liver extracts from naphthenic acids-fed rats yielded a small hump in the RICs (*m/z*=267) between 15 to 20 min consistent with the presence of naphthenic acids in full mass range scan mode (Figure 4.9B) or narrow scan mode (Figure 4.9C). The detection of naphthenic acids in liver tissue from rats fed suggests that these acids were absorbed through the gastrointestinal track and transported into the liver. These results are consistent with the findings of Firriolo et al. (1999), who reported that cobalt naphthenate was dissociated at the pH of the stomach and possible absorbed into liver of Fisher rats. Also, with the findings of Rogers et al. (2002a), who reported that the liver was the target organ of naphthenic acids toxicity in acute exposure experiments using Wistar rats.

In addition, three liver samples from rats in the control group A that were not fed naphthenic acids, were extracted and analyzed by GC-MS. All the liver extracts from the control group chosen were analyzed in full mass range scan mode and no naphthenic acids were detected in any of the control samples analyzed as shown in Figure 4.10A. Liver sample from rat #5 was re-analyzed in narrow mass range scan mode, and similarly to full mass range scan mode, the RIC chromatogram did not showed a hump in the retention time window of naphthenic acids (Figure 4.10B).

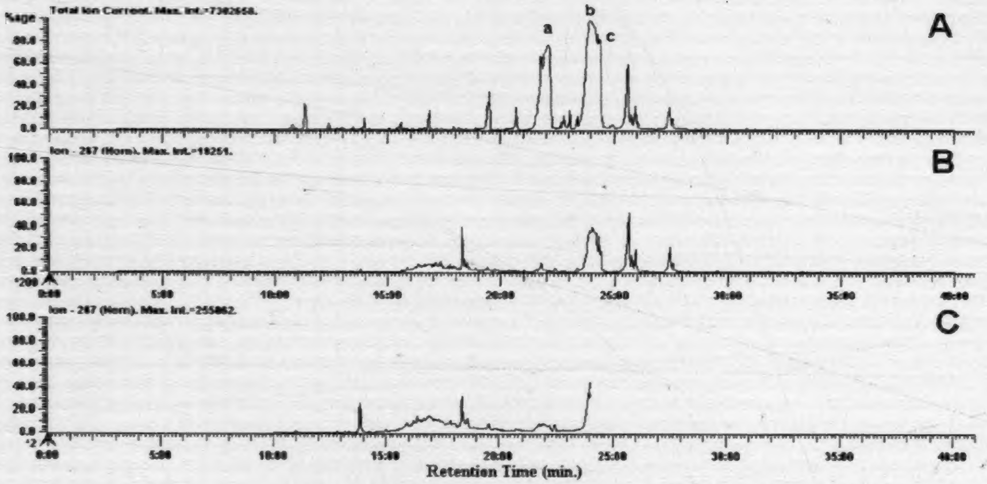


Figure 4.9 (A) TIC of the extract of 5 g of liver tissue from rat #12 fed with 300 mg of Merichem naphthenic acids kg^{-1} and scarified 6 h after ingestion; (B) RIC for the nominal *m/z*=267 yielding a small hump between 15 to 20 min indicating the presence of naphthenic acids in full mass range scan mode and (C) in narrow mass range scan mode. Peak identities are the same as in Figure 4.1.

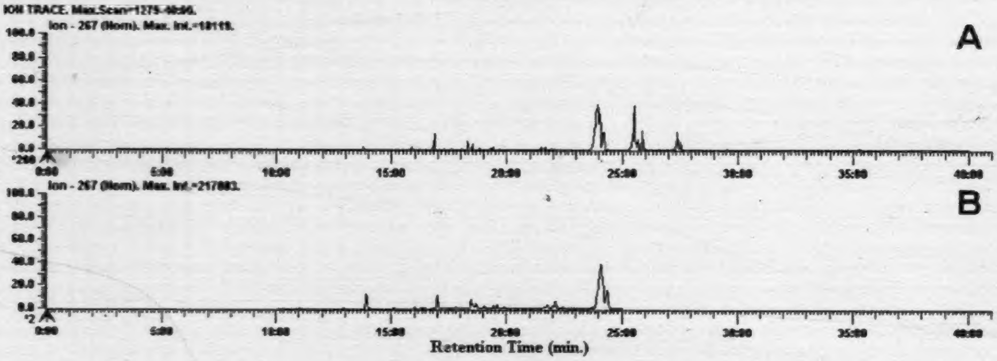


Figure 4.10 RICs from the extract of 5 g of liver from rat #5 (control group A) not fed naphthenic acids. (A) RIC for the nominal *m/z*=267 did not yield a hump in the retention time window of naphthenic acids in full mass range scan mode and (B) in narrow mass range scan mode.

4.5.5 GC-HRMS Analyses of Rat Liver

Four liver extracts were chosen for GC-HRMS analyses using SIM with the exact mass of 267.1780, corresponding to $C_{15}H_{27}O_2Si^+$ from *tert*-butyldimethylsilyl esters of naphthenic acids with the formula $C_{13}H_{22}O_2$. These liver extracts included: two livers from fed rats experiments, that is one fed with naphthenic acids (rat #16, Group B) and one not fed with naphthenic acids (rat #1, Group A), and two liver extracts from spiked samples, that is one liver extract spiked 0.5 µg g^{-1} and one not spiked. Figure 4.11 showed the SIM chromatogram of the liver extract from rat #16 where a defined hump that eluted with retention time from 17 to 20 min was observed in the chromatogram consistent with the presence of naphthenic acids. SIM results from the rat liver spiked with 0.5 µg g^{-1} also showed a hump from 17 to 20 min (data not shown). Additionally, not humps were seen in the SIM chromatograms of a control sample of rat liver #1 (not fed with naphthenic acids) or a liver extract without added naphthenic acids (data not shown). Although, these results were in agreements with the results form the GC-LRMS, no significant improvement was obtained in GC-HRMS analyses, due to co-extractive fatty acids present in the biological material.

Alternatively to analyze the liver or plasma extracts by HR, these extracts can be diluted to reduce matrix effects or analyzed in a narrow mass range instead of SIM, which will result in loss of selectivity and sensitivity. One of the advantages of the HR analysis is their high selectivity and sensitivity, as none of these characteristics were accomplished in HR analyses of biological extracts due to matrix effects, no further samples where analyzed by GC-HRMS. In general, HR analyses were not well suited for resolving interference from co-extractive materials present in the liver tissue extracts.

Overall, with the instrumentation available, SIM analyses are more difficult than LRMS analyses.

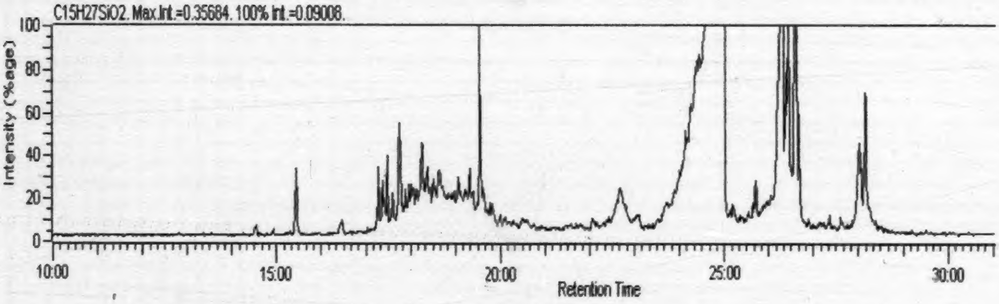


Figure 4.11 SIM for the ion with exact mass of 267.1780 of the extract of 5 g of rat liver sample from rat # 16 fed with 300 mg Merichem naphthenic acids kg^{-1} body weight and scarified 6 h after oral ingestion

4.3.6 Overall Discussion

A summary of the results obtained for the detection of naphthenic acids in plasma samples and liver samples is given in Table 4.1. In this table, a sample was assigned as "+", when the RIC for the nominal mass of 267 showed a hump with retention time between 15 to 20 min. If the sample did not showed any hump in that retention time window, the sample was assigned as "–".

Results from plasma extracts were based on narrow mass range scanning and results from liver extracts were based on both full and narrow mass range scanning since the same results were acquired in either mode. The samples of rat liver analyzed in this study were chosen to match the plasma samples provided in order to correlate the results. All the liver extracts from rats fed with naphthenic acids analyzed by GC-LRMS showed a small hump in the RICs for the *m/z* =267 with retention time between 15 to 20 min indicative of the presence of naphthenic acids (Table 4.1). In the plasma extracts results only one of the samples from rats fed with naphthenic acids showed a small hump in the RIC for the nominal mass of 267 indicating the presence of naphthenic acids.

Table 4.1 Summary of the detection of naphthenic acids in rat liver and plasma samples.

Plasma Samples (1 mL)	Detection of naphthenic acids	Liver Samples (5 g)	Detection of naphthenic acids
	Samples spiked with Merichem naphthenic acids		
1 μg mL^{-1}	+	1 μg g^{-1}	+
0.5 μg mL^{-1}	+	0.5 μg g^{-1}	+
Not spiked	–	Not spiked	–
	Samples from rats fed Merichem naphthenic acids		
Rat #12	+	Rat #12	+
Rat #13	–	Rat #13	+
Rat #15	–	Rat #14	+
Rat #16	–	Rat #16	+
	Samples from rats not fed naphthenic acids		
Rat #4	–	Rat # 4	–
Rat #5	–	Rat #5	–
Rat #6	–	Rat #6	–

In this study, it was assumed that naphthenic acids will be present in serum or plasma as free fatty acids rather than being chemically bonded with complex lipids, thus methods for extraction of free fatty acids were investigated in serum or plasma samples. However, most of the samples provided from the rat exposure experiments were whole blood. Thus, some attempts were made to extract naphthenic acids from blood samples using a similar procedure as the one reported in Section 4.2.7 for the liver tissues. In this procedure, total lipids were extracted from 1 mL of heparinized blood sample spiked with 5 µg of Merichem naphthenic acids. During the extraction, excessive emulsions developed, making it difficult to collect the organic phase and reducing the extraction efficiency. After three consecutive extractions, the pooled extracts containing the total lipids were cleaned up with a SAX SPE column, followed by derivatization of the carboxylic acids with MTBSTFA and GC-LRMS analysis. RICs for the nominal mass of 267 did not show a hump as indicative of the presence of naphthenic acids between 15 to 20 min, but abundance of peaks were observed resulting from the extraction of total lipids, whereas in plasma samples, as the extraction procedure reported in Section 4.2.6 only extract free fatty acids and naphthenic acids a lower detection of naphthenic acids can be accomplished. Based on these results, only rat plasma samples were analyzed in this study.

The detection of naphthenic acids in biological samples require a selective and sensitive method of analysis, however, the abundance of fatty acids that are co-extracted with naphthenic acids and the complexity of the mixture makes it difficult to accomplish great sensitivity. Thus, in order to improve the instrumental detection of naphthenic acids in tissues and plasma, the extracts were acquire in narrow range of masses instead of in a full range of masses. In narrow mass range, the most abundant fatty acids that elute from the GC column after 22 min were avoided because the chromatogram is acquired from 10 to 23 min. In addition, these new settings allowed to increase the gain in the MS or to increase the volume of sample injected into the GC without saturate the MS, but these two parameters cannot be obtained by full mass scan, because an increase of gain or an increase in the volume of sample injected into the GC-MS would saturate the MS and give inaccurate peaks.

Figure 4.1 showed a comparison between a plasma extract analyze in full mass range mode (Figure 4.1B) and in narrow mass range mode (Figure 4.1C). This comparison clearly showed that the RIC from the plasma sample analyzed in narrow mass range mode (Figure 4.1C) produced an evident hump that is not observed in the RIC of a similar plasma extract analyzed in full mass range mode (Figure 4.1B), unless the chromatogram is enlarged. Unfortunately, as this is a qualitative method of analyses based on the finding of a hump in the RIC for *m/z* =267 in a specific retention time, concentrations of naphthenic acids detected in both samples cannot be determined. Similar to plasma extracts, liver extracts samples were analyzed in full mass range and narrow mass range and the results from these evaluations showed that narrow mass scan mode produce "evident" chromatograms that are not always acquired with full scan mode.

Several studies have evaluated the toxic effects of naphthenic acids in aquatic organisms (Dokholyan and Magomedov 1983) and mammals (Rogers et al. 2002a), but the lack of methodologies to extract and to detect naphthenic acids in tissues have limited toxicity studies to measure the absorption and the distribution of these compounds in biological organisms. For example, Firriolo et al. (1999) evaluated the absorption of cobalt naphthenates after an oral administration in Fisher rats. Their results showed that the highest concentration of cobalt detected was in the liver and kidneys, also they reported that the maximum peak concentration of cobalt in plasma occurred 4.3 h after ingestion. However, the naphthenate moiety was not investigated due to lack of suitable extraction techniques, but they believe that cobalt naphthenate is rapidly absorbed after an oral administration and about 20% is excreted in the urine.

Rogers et al. (2002a) suggested that the toxicity observed in rats that were orally fed naphthenic acids was induced by naphthenic acids metabolites rather than parent compounds due to the induction of enzymes from cytochrome P450, which are enzymes involved in the metabolism of endogenous compounds. In that study, the liver was the target organ for naphthenic acids toxicity, however, the concentrations of naphthenic acids or their metabolites was not determine. More recently, Nero et al. (2006) studied the histopathology of livers and gills from fish exposed to process-affected waters containing naphthenic acids. Fish liver showed hepatocellular degeneration and gill tissues showed epithelial cell necrosis, but due to the complexity of the oil sands constituents these results cannot be traced to a specific compound. They also reported the induction of the enzymatic activity by cytochrome P450.

Most toxicity studies have shown that the liver is target organ for naphthenic acids. Thus, the methods described in this chapter allowed to detect naphthenic acids in liver tissue and plasma from Wistar rats, but with the appropriate modifications these methods can be applied to other tissues and to other species. For example, Young et al. (2007) applied this method to detect naphthenic acids in fish fillets and guts, and further they may apply to fish liver.

4.4 Conclusions

A qualitative method of analysis based on the detection of the *tert*-butyldimethylsilyl derivatives of naphthenic acids by GC-LRMS was developed to specifically detect naphthenic acids in liver tissues and blood plasma from rats fed with Merichem naphthenic acids. A lower detection of naphthenic acids of 0.5 μg g^{-1} for liver samples and 0.5 μg mL^{-1} for plasma samples were achieved when the extracts were analyzed in narrow mass range. Although a detection limit was established in this study, this is a strictly qualitative method of analyses. Small amounts of naphthenic acids were detected in liver extracts from rats fed naphthenic acids and one plasma extract from rat fed (#12, Table 4.1). These results suggest that parent naphthenic acids can be taken up by the liver and perhaps distributed to the other tissues through the blood stream.

4.5 Literature Cited

Brient, J.A., Wessner, P.J. and Doly, M.N. 1995. Naphthenic acids. In: Kroschwitz, J.I. (Ed.). Encyclopedia of Chemical Technology 4th edition, Vol. 16. John Wiley and Sons Inc., New York. pp. 1017-1029.

CONRAD. 1998. Naphthenic acids background information discussion report. Canadian Oil Sands Network for Research and Development, Environmental Aquatic Technical Advisory Group.

Clemente, J.S. and Fedorak, P.M. 2004. Evaluation of the analyses of tert-butyldimethylsilyl derivatives of naphthenic acids by gas chromatography-electron impact mass spectrometry. Journal of Chromatography A, 1047: 117-128.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology, 38: 1009-1016.

Dokholyan, B.K. and Magomedov, K.A. 1984. Effect of sodium naphthenate on survival and some physiological-biochemical parameters of some fishes. Journal of Ichthyology, 23: 125-132.

Firriolo, J.M., Ayala-Fierro, F., Sipe, G.I. and Carter, D. 1999. Absorption and disposition of cobalt naphthenate in rats aafte a single oral dose. Journal of Toxicology and Environmental Health, Part A, 58: 383-395.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research, 36: 2843-2855.

Jivraj, M.N., MacKinnon, M. and Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. In: Syncrude Analytical Methods Manual. Research Department, Syncrude Canada Ltd., Edmonton. AB.

Jones, D.M., Watson, J.S., Meredith, W., Chen, M. and Bennett, B. 2001. Determination of naphthenic acids in crude oils using nonaqueous ion exchange solid-phase extraction. Analytical Chemistry, 73: 703-707.

Kamaluddin, M. and Zwiazek, J.J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. Tree Physiology, 22: 1265-1270.

Kasperski, K.L. 1992. A review of properties and treatment of oil sands tailings. AOSTRA Journal of Research, 8: 11-52.

Lawson, A.M. 1989. Mass Spectrometry. Walter de Gruyter and Co, Berlin New York

Leung, S.S., MacKinnon, M.D. and Smith, R.E.H. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry, 20: 1532-1543.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. 2003. Electrospray-mass spectrometric analysis of reference carboxylic acids and Athabasca oil sands naphthenic acids. Analytical Chemistry, 75: 6394-6400.

Luque-Garcia, J.L. and Neubert, A. 2007. Sample preparation for serum/plasma profiling and biomarker identification by mass spectrometry. Journal of Chromatography A, 1153: 259-276.

MacKinnon, M.D. and Boerger, H. 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Canadian Journal of Water Pollution and Research 21, 496-512.

MacKinnon, M.D. 1989. Development of the tailings pond at Syncrude's oil sands plant: 1978-1987. AOSTRA Journal of Research, 5: 109-133.

Mehta, A., Oeser, A.M. and Carlson, M.G. 1998. Rapid quantification of free fatty acids in human plasma by high-performance liquid chromatography. Journal of Chromatography B, 719: 9-23.

Morris, P.C., Gallimore, P., Handley, J., Hide, G., Haughton, P. and Blank, A. 2005. Full-fat soya for rainbow trout (*Oncorhynchus mykiss*) in freshwater: Effects on performance, composition and flesh fatty acid profile in absence of hind-gut enteritis. Aquaculture, 147-161.

Murray, K.K., Boyde, R.K., Eberlin, M.N., Langley, G.J., Li, L. and Naito, Y. 2006. IUPAC Standard Definitions of Terms relating to Mass Spectrometry. IUPAC

project 2003-056-2-500.
http://www.msterms.com/docs/IUPAC_MS_Terms_Second_Draft.pdf

Nero, V., Farwell, A., Lister, A., Van Der Kraak, G., Lee, L.E.J, Van Meer, R., MacKinnon, M.D. and Dixon, D.G. 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected waters. Ecotoxicology and Environmental Safety, 63: 365-377.

Nix, P.G.and Martin, R.W. 1992. Detoxification and reclamation of Suncor's oil sands tailings ponds. Environmental Toxicology and Water Quality, 7: 171-188.

Rudzinski, W.E., Oehlers, L. and Zhang, Y. 2002. Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy and Fuels, 16: 1178-1185.

Schramm, L.L., Stasiuk, E.N. and MacKinnon, M. D. 2000. Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings process in Schramm, L. L., editor. Surfactants: Fundamentals and applications in the petroleum industry. Cambridge University Press, Cambridge.

Scott, A.C., MacKinnon, M.D. and Fedorak, P.M. 2005. Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable that commercial naphthenic acids. Environmental Science and Technology, 39: 8388-8394.

Slavcheva, E., Shone, B. and Turnbull, A. 1999. Review of naphthenic acid corrosion in oil refining. British Corrosion Journal, 34: 125-131.

Rogers, V.V., Wickstrom, M., Liber, K. and MacKinnon, M.D. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands. Toxicological Sciences, 66: 347-355.

Tissot, B.P., Welte, D.H. 1978. Petroleum Formation and Occurrence. Springer-Verlag, New York.

van den Heuvel, M.R., Power, M., MacKinnon, M.D., Van Meer, T., Dobson, E.P.and Dixon, D.G. 1999a. Effects of oilsands related aquatic reclamation on yellow perch (*Perca flavescens*). I. Water quality characteristics and yellow perch physiological and population responses. Canadian Journal of Fisheries and Aquatic Science, 56: 1213–1225.

van den Heuvel, M.R., Power, M., MacKinnon, M.D. and Dixon, D.G., 1999b. Effects of oilsands related aquatic reclamation on yellow perch (*Perca flavescens*). II. Chemical and biochemical indicators of exposure to oil sands related waters. Canadian Journal of Fisheries and Aquatic Science, 56: 1226–1233.

van den Heuvel, M.R., Power, M., Richards, J., MacKinnon, M.D. and Dixon, D.G. 2000. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to oil sands mining-associated waters. Ecotoxicology and Environmental Safety, 46: 334–341.

Verbeek, A.G., Mackay, W.C. and MacKinnon, M.D. 1994. A toxicity assessment of oil sands wasterwater: a toxic balance. Canadian Technical Report of Fisheries and Aquatic Science, 1989; 196-207.

Young, R. F., Orr, E.A., Gross, G.G. and Fedorak, P.M. 2007. Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water. Chemosphere, 68: 518-527.

5. General Discussion and Suggestions for Future Research

5.1 Discussion

The main objective of this research was to assess whether the gas chromatography-mass spectrometry (GC-MS) method developed by St. John et al. (1998) and, extensively used by this research group to characterize diverse mixtures of naphthenic acids and in biodegradation studies, can specifically detect naphthenic acids among the naturally occurring fatty acids that are usually present in samples such as river water, plants, and animal tissues.

To date, most of the common methods described to measure naphthenic acids concentrations in water samples either measure the acidity of the sample as TAN (Brient et al.1995, Slavcheva et al. 1999) or measure the carboxylic acid functional group as FTIR method (Jivraj et al. 1995) or HPLC method (Clemente et al. 2003, Yen et al. 2004), therefore those methods are not specific to naphthenic acids because other acidic compounds present in petroleum samples or fatty acids accounts in both determinations. Other methods such as GC-FID (Jones et al. 2002), ESI-MS (Headley et al. 2002, Lo et al. 2003) and most recently HPLC/QTOF-MS (Bataineh et al. 2006) have been used to measure concentrations of naphthenic acids, but they either require expensive equipment and specialized training that is not always available in conventional laboratories or have low sensitivity.

In order to accomplish the objective of this study, the first exercise was to examine in detail the three-dimensional plots of naphthenic acids either from commercial or from oil sands samples including tailings, process-affected waters, surface waters, groundwaters and ores previously characterized by GC-MS in different studies. Due to the great diversity of naphthenic acids with different carbon and *Z* numbers, it was assumed that certain naphthenic acids with specific combinations of *n* and *Z* would be very common and abundant in the different naphthenic acids mixtures that these ions would provide a key to distinguish naphthenic acids from fatty acids.

A table with about 45 mixtures of naphthenic acids was produced showing which ions are characteristic of *tert*-butyldimethylsilyl derivatives of naphthenic acids. Four ions with the nominal masses of 265, 267, 279 and 281 that corresponds to naphthenic acids derivatives with the formula $C_{13}H_{20}O_2$, $C_{13}H_{22}O_2$, $C_{14}H_{22}O_2$ and $C_{14}H_{24}O_2$, respectively, were selected for reconstructed ion chromatograms (RICs, Murray et al. 2006). From these ions, the ion with the nominal mass of 281 was excluded because previous studies of Clemente and Fedorak (2004) demonstrated that the abundance of this ion is influenced by GC column bleed. Thus, the ions with the nominal masses of 265, 267 and 279 were selected to specifically detect naphthenic acids by GC-MS based on RICs. These ions were selected not only because they were relatively abundant in many mixtures of naphthenic acids, but also because naturally occurring fatty acids with 13 carbons and two or three unsaturations and 14 carbons with three unsaturations are not common in biological materials, and if they were present, they would not elute from the GC column as unresolved humps as naphthenic acids due to the abundance of isomers with the same *n* and *Z* families.

Additionally, the RICs of each of these ions yield a characteristic hump in a specific retention time depending on the ion acquired. For example, ions with the nominal mass of 267 are usually acquired between 15 to 20 min as showed in Figure

2.2C, in contrast with ions with the nominal mass of 279, which are usually acquired between 17 to 23 min as showed in Figure 2.2D. Thus, the detection of naphthenic acids in this study is based on the detection of a hump in the RIC for the specific ions at retention time between 15 to 23 min.

There is ample evidence suggesting that naphthenic acids have different composition based on carbon numbers and *Z* families according to the source (Holowenco et al. 2002, Clemente et al. 2004, Scott et al. 2005, Bataineh et al. 2006). As a consequence, the present research evaluated the GC-MS method using commercial Merichem naphthenic acids and oil sands naphthenic acids extracted from West-In Pit (WIP). When any of these mixtures were spiked into water samples, no difference in the detection of these acids was observed in either mixture used and the same detection limit was accomplished (Chapter 2). However, orally fed Wistar rats were only dosed with naphthenic acids from Merichem acids due to the lack of oil sands naphthenic acids extract with the sufficient purity and quantity required to orally feed 10 rats at 300 mg kg^{-1} body weight. Similarly, plant tissues were only spiked with WIP naphthenic acids because of time constraint to continue developing the method.

Before the GC-MS method could be applied to the analyses of water samples, animal tissues or plant tissues, it was necessary establish that naphthenic acids could be extracted from the sample matrix and derivatized. To do this, a variety of methods were investigated to extract free fatty acids and naphthenic acids from each matrix assuming that naphthenic acids would be present in water samples, animal tissues or plant samples in free form rather than be bound to other molecules. Thus, an extraction procedure for water samples is reported in Chapter 2, for plant tissues is reported in Chapter 3 and for liver tissues and plasma in Chapter 4.

The application of the GC-MS method to 23 water samples collected from different locations in Canada showed encouraging results. For example, no naphthenic acids were detected in 11 water samples collected far away from the oil sands or to the knowledge of the author were not in contact with naphthenic acids. These results helped to verify that the RICs showed no humps near 20 min retention time that could be falsely assigned as naphthenic acids.

In addition, it is remarkable that naphthenic acids could be detected at a concentration of 10 μg L^{-1} in water samples. However, six water samples including one sample from a refinery treated effluent, one sample from a storage tank, two samples of oil field produce waters, two domestics well waters samples and a yellow water sample collected from a pond located in Edmonton, showed the limitations of the GC-LRMS method to detect naphthenic acids. Initially, the six water samples mentioned above showed the presence of naphthenic acids based on GC-LRM analysis, but when the six extracts were brominated, to eliminate compounds in the extracts with double bonds that could be incorrectly assigned as naphthenic acids, the results showed that the characteristics humps of naphthenic acids disappeared after bromination in the water from an oil storage tank and from a colored pond, indicating that some compounds in the extracts were incorrectly assigned as naphthenic acids ions by GC-LRMS, when they were not naphthenic acids.

Therefore, it was necessary to confirm the results with more powerful techniques such as gas chromatography-high resolution mass spectrometry (GC-HRMS) using single ion monitoring (SIM) for the exact mass of 267.1780, which results were in agreement with the reported by GC-LRMS (Table 2.2) in the two brominated extracts mentioned above. However, SIM analyses of the brominated extracts of the refinery water sample and oilfield produce water 2, the results were in disagreement with the previous results obtained by GC-LRMS. It is possible that the extensive clean up steps before the extraction of these samples, pH of the sample and the small volume of sample use may affected the detection of the characteristic ions by GC-HRMS.

Surprisingly, the non-brominated and brominated extracts from two domestic wells collected at east and northwest of Edmonton showed the presence of naphthenic acids analyzed by GC-LRMS and GC-HRMS. However, there is not evidence that these two well water samples would be in contact with petroleum acids, thus these results indicates the presence of compounds that are undistinguishable from naphthenic acids.

Although the GC-LRMS method has some limitations for the detection of naphthenic acids, this method has a lower detection limit and can be applied to a wide variety of water samples. In addition, bromination of the extract and SIM analysis improve the confidence of the method.

A method to extract naphthenic acids from cattails plants was developed to evaluate if naphthenic acids can be detected and if they have accumulated in the tissues of cattails growing in naphthenic acids-containing wetlands located at the oil sands operations. During the method development, the abundance of pigments from cattails extracts and the complex GC-MS chromatograms acquired complicate the detection of lower concentrations of naphthenic acids resulting in a laborious method of extraction. As a consequence, only a few cattails plants spiked with West-In pit naphthenic acids were evaluated and the results are reported in Chapter 3 indicating that no compounds that produce humps were detected over the background of fatty acids from cattails extracts as shown in Figure 3.2. In addition, the naphthenic acids were only detected in spiked samples at concentration > 5 μg g^{-1} tissue, as a result of the laborious extraction procedure.

Also, due to time constrains to complete these research, Rozlyn Young perfected the cattail extraction procedure (See Appendix A for report) and applied this GC-MS method to cattails extracts from plants collected in the late summer of 2005 from pond #5 containing 24 mg naphthenic acids L^{-1} in the free water zone. Based on RIC for the nominal mass of 267, no naphthenic acids were detected in cattails leaves, but only in the roots indicating that these petroleum acids are primarily located in the roots and not uploading to the xylem to be translocated into the leaves as suggested by Kamaluddin and Zwiazek (2002) for aspen seedlings. It is possible that these results were influence by the high salinity of the pond and the low concentration of naphthenic acids.

The testing of the GC-LRMS method to liver tissues extracts from Wistar rats exposed to a single dose of 300 mg Merichem naphthenic acids kg^{-1} body weight (Chapter 4) over 6 h showed that naphthenic acids were detected based on RIC for the nominal mass of 267 in all the liver samples from exposed rats analyzed and summarized in Table 4.1. In addition, no naphthenic acids were detected in the liver samples from not exposed rats, these results confirmed that there are no compounds in the profile of extracted fatty acids that could be falsely assigned as naphthenic acids.

In this study, the liver was the only organ tested from fed rats because previous work by Rogers et al. (2002a) suggested that the liver was the target organ for naphthenic acids toxicity in both acute and subchromic experiments. Additionally, plasma samples from fed rats were examined to determine if naphthenic acids could be detected. Similarly to liver samples, no naphthenic acids were detected in rats fed with tap water; however, naphthenic acids were detected based on the RIC for the nominal mass of 267 in one of the plasma samples from fed rats. This result was not further investigated due to the limited volume of blood samples provided.

The results reported in Chapter 4 for the detection of naphthenic acids in liver tissues and plasma based on RIC for the ion the nominal mass of 267 clearly indicates the ability of the GC-LRMS method to detect naphthenic acids in biological samples. However, it is possible that the short period of time between the exposure and sacrifice of the rats, and the mixture used influence the detection of naphthenic acids in the liver tissues and plasma samples.

In conclusion, the results from this study provided evidence that the GC-MS method of St. John et al. (1998) can be used to detect naphthenic acids based on characteristic *tert*-butyldimethylsilyl derivatives. The GC-MS analysis of samples spiked with naphthenic acids or in contact with naphthenic acids showed that these compounds were selectively detected over the profile of fatty acids in water samples, plasma, liver tissues and plant tissues. Although, some limitations of the method were observed in water samples that required further bromination and HR analysis. This method can be useful to evaluate the impact of these acids in the environment.

5.2 Suggestions for Future Research

The GC-MS method presented in this research to specifically detect naphthenic acids is strictly qualitative and is based on the detection of a *tert*-butyldimethylsilyl derivative with the nominal mass of 267, which is characteristic and abundant in many naphthenic acids mixtures. Although this GC-MS method yields useful information of the detection of naphthenic acids in water, liver tissues, plasma and plants tissues, the data are not quantitative and further developments of the analytical method, including the addition of a suitable internal standard and preparation of a calibration curve will be needed before is possible to use the method described here as a means to monitor naphthenic acids uptake by different species.

There are also further research needs regarding the absorption and distribution of naphthenic acids in mammals. Ideally, GC-MS analyses of the extracts of the rat tissues gathered by Rogers et al. (2002a) in a toxicity study would give more significant results. However, these samples were not available, so new exposure experiments are required using oil sands naphthenic acids instead of commercial naphthenic acids, which are known to be more recalcitrant than commercial mixtures. In addition, extending the time of exposure and collecting additional tissues such as kidney, heart or intestine will yield a new insight of the distribution and possible absorption of naphthenic acids that help to understand the toxicity of these compounds.

New evidence showed that using quadrupole MS provide more sensitive detection than magnetic sector MS (Rozlyn Young, personal communication), this advantage would be valuable in further studies to establish the detection limit of naphthenic acids in complex matrices such as plants tissues that was not addressed in this thesis.

5.3 Literature Cited

Bataineh, M., Scott, A.C., Fedorak, P.M. and Martin, J.M. 2006. Capillary HPLC/QTOF-MS for characterizing complex naphthenic acid mixtures and their microbial transformation. Analytical Chemistry, 78: 8354-8361.

Brient, J.A., Wessner, P.J. and Doly, M.N. 1995. Naphthenic acids. In: Kroschwitz, J.I. (Ed.). Encyclopedia of Chemical Technology, 4th ed. Vol. 16. John Wiley and Sons, New York, NY, pp. 1017-1029.

Clemente, J.S. and Fedorak, P.M. 2004. Evaluation of the analyses of *tert*-butyldimethylsilyl derivatives of naphthenic acids by gas chromatography-electron impact mass spectrometry. Journal of Chromatography A, 1047: 117-128.

Clemente, J.S., Yen, T.-Y. and Fedorak, P.M. 2003. Development of a high performance liquid chromatography method to monitor the biodegradation of naphthenic acids. Journal of Environmental Engineering and Science, 2: 177-186.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology, 38: 1009-1016.

Headley, J.V., Peru, K.M., McMartin, D.W. and Winkler, M. 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. Journal of AOAC International, 85: 182-187.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research, 36: 2843-2855.

Jivraj, M.N., MacKinnon, M. and Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Analytical Methods Manual. 4th ed. Research Department, Syncrude Canada Ltd., Edmonton, Canada.

Jones, D.M., Watson, J.S., Meredith, W., Chen, M. and Bennett, B. 2001. Determination of naphthenic acids in crude oils using nonaqueous ion exchange solid-phase extraction. Analytical Chemistry, 73: 703-707.

Kamaluddin, M. and Zwiazek, J.J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. Tree Physiology, 22: 1265-1270.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. 2003. Electrospray-mass spectrometric analysis of reference carboxylic acids and Athabasca oil sands naphthenic acids. Analytical Chemistry, 75: 6394-6400.

Murray, K.K., Boyde, R.K., Eberlin, M.N., Langley, G.J., Li, L., Naito, Y., 2006. IUPAC Standard Definitions of Terms relating to Mass Spectrometry. IUPAC project 2003-056-2-500. http://www.msterms.com/docs/IUPAC_MS_Terms_Second_Draft.pdf

Rogers, V.V., Wickstrom, M., Liber, K., MacKinnon, M.D. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands. Toxicological Sciences, 66: 347-355.

Scott, A.C., MacKinnon, M.D. and Fedorak, P.M. 2005. Naphthenic acids in Athabasca oil sands tailings water are less biodegradable than commercial naphthenic acids. Environmental Science and Technology, 39: 8388-8394.

Slavcheva, E., Shone, B. and Turnbull, A. 1999. Review of naphthenic acid corrosion in oil refining. British Corrosion Journal, 34: 125-131.

St. John, W.P., Rughani, J., Green, S.A. and McGinnis, G.D. 1998. Analysis and characterization of naphthenic acids by gas chromatography electron impact mass spectrometry of *tert*-butyldimethylsilyl derivatives. Journal of Chromatography A, 807: 241-251.

Yen, T.-W., Marsh, W.P., MacKinnon, M.D. and Fedorak, P.M. 2004. Measuring naphthenic acids concentrations in aqueous environmental samples by liquid chromatography. Journal of Chromatography A, 1033: 83-90.

Appendices

Appendix A: Extraction of Cattails for Naphthenic Acids Analyses

R.F. Young and P.M. Fedorak

Abstract

Naphthenic acids are a mixture of acyclic and cycloaliphatic carboxylic acids present in the Athabasca oil sands near Fort McMurray, Alberta. The process of extracting bitumen from the oil sands produces large volumes of water high in naphthenic acids. These oil sands process-affected waters are stored on site in large settling ponds because they cannot be released into the environment. Naphthenic acids are the most toxic component of the process-affected water. Cattails collected from process-affected wetlands were extracted and analyzed by gas chromatography-mass spectrometry (GC-MS) with reconstructed ion chromatograms (RICs) for nominal *m/z* = 267 to specifically detect naphthenic acids. In a test extraction, a sample of a cattail leaf collected from the Edmonton, Alberta area was spiked with 5 μg of commercial naphthenic acids/g. Naphthenic acids were detected in the spiked cattail leaf sample by GC-MS in the RIC for *m/z* = 267, confirming that the extraction method could be applied to plant tissues. Cattails collected from a process-affected wetland were separated into lower leaf, middle leaf and upper leaf sections, and combined rhizome and roots. Naphthenic acids were not detected in the leaf samples. The rhizome and roots from a second cattail collected from the process-affected wetland were washed with a Na_2CO_3 solution, and naphthenic acids were detected in the wash solution, in the roots, and in the rhizome.

Introduction

Naphthenic acids are a complex mixture of acyclic and cycloaliphatic carboxylic acids defined by the chemical formula $C_nH_{2n+Z}O_2$ (Brient et al., 1995), where n indicates the carbon number and *Z* is zero or a negative, even integer whose absolute value divided by 2 gives the number of rings, fused or bridged, in the compound. The biodegradation of petroleum in the Athabasca oil sands near Fort McMurray, Alberta, Canada produced naphthenic acids in the deposits (Tissot and Welte, 1978) and the naphthenic acids are released into the aqueous phase from oil sands extraction at alkaline pH.

The aqueous slurry, or tailings, leftover from oil sands extraction contains water, sand, clay, naphthenic acids, residual bitumen and inorganic and organic constituents. Tailings are stored on site in large settling ponds. As the solids separate from the tailings, forming mature fine tails (MFT), water is released creating a surface water layer with low solids. This is called process-affected water and it is recycled back to the extraction plant. These process-affected waters can contain 20-120 mg naphthenic acids/L (Holowenko et al., 2002).

Naphthenic acids are the most toxic component of oil sands process-affected water (MacKinnon and Boeger, 1986; Verbeek et al., 1994). Kamaluddin and Zwiazek (2002) found that aspen seedlings were inhibited by naphthenic acids. They observed decreased root water flux, root respiration, leaf stomatal conductance, photosynthesis, chlorophyll concentration, and leaf size in aspen seedlings growing in the presence of commercial naphthenic acids. Naphthenic acids were found in the xylem of the aspen seedlings when the roots were placed in a pressure chamber, suggesting that the naphthenic acids were able to move from the aqueous solution surrounding the roots into the plant (Kamaluddin and Zwiazek, 2002). The uptake of organics by plants is driven by diffusion and can be estimated using log K_{ow} values, the octanol:water partition coefficient. Organics with log K_{ow} values between 0.5 and 3 are hydrophobic enough to move into plant membranes and hydrophilic enough to travel through plant fluids (Pilon-Smits, 2005). Naphthenic acids are a mixture of compounds, with log K_{ow} values between 2 and 5 (Havre et al., 2003).

Oil sands process-affected waters cannot be released into the environment, however seepage from the tailings ponds created pools of water and wetland ecosystems became established in the presence of oil sands effluent. Crowe et al. (2001) studied the effects of oil sands effluent on cattails (Figure 1) collected from wetlands on the Suncor Inc. site. The cattails had been growing in the presence of high Na^+, SO_4^{2-} and Cl^-, and naphthenic acids. In microcosm studies, the plants exposed to oil sands effluent showed higher levels of apparent photosynthesis than controls. Although Crowe et al. (2001) found that cattails were well adapted to growth in oil sands effluent; they suggested that more studies are necessary to determine long-term survival of plants exposed to oil sands process-affected water.

The objective of this study was to determine if naphthenic acids could be extracted from cattails spiked with a commercial preparation of naphthenic acids and detected using analysis by gas chromatography-mass spectrometry (GC-MS). Merlin et al. (2005, 2007) demonstrated that naphthenic acids can be specifically detected in natural waters, animal serum, and plant tissues using reconstructed ion chromatograms (RICs) for nominal *m/z* = 267 from the GC-MS analyses of derivatized extracts. Cattails collected from a process-affected wetland were similarly extracted and analyzed for naphthenic acids.

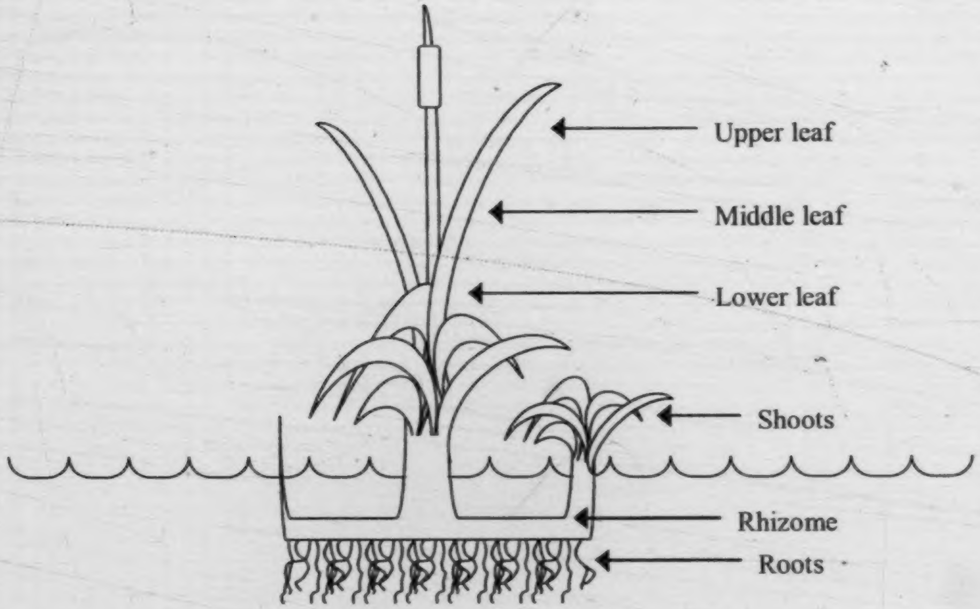


Figure 1 Diagram of a cattail (adapted from Sojda and Solberg, 1993). The rhizome is an underground steam that grows horizontally and sends out roots and shoots.

Materials and Methods

Naphthenic acids and sources of cattails

Refined Merichem naphthenic acids were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX). Cattails for use as non-process affected wetland controls were collected near Edmonton, Alberta, Canada. Cattails collected from a process-affected wetland were harvested in June 2005 from Syncrude's Test Pond 5, a site constructed in 1989 containing MFT capped with tailings pond water (Siwik et al.,

2000) and estimated to contain 24 mg naphthenic acids/L. Cattails from Syncrude's Pond 5 were approximately 105 cm from rhizome to leaf tip. Individual typha (whole plants) were sampled into plastic bags. All cattails were stored at -20°C prior to extraction and analysis.

Extraction of cattails for naphthenic acids

The extraction method was adapted from the procedures reported by Bernárdez et al. (2005) for the analysis of free fatty acids from fish. Previous work using fish has shown that the detection limit for this method is approximately 1 µg of naphthenic acids/g fish (Young et al., 2006, 2007). All glassware used for cattail extraction was thoroughly cleaned and rinsed with dichloromethane prior to use. To 5 g of control cattail leaves cut into 1 cm^2 pieces in a 250-mL Erlenmeyer flask, 25 µg of Merichem naphthenic acids was added, to give a concentration of 5 µg/g of cattail. Then 100 mL of a 2:1 chloroform:methanol mixture was added and this was homogenized for 2 min using an Ultra Turrax (Model T18 basic, IKA Works Inc., Wilmington, NC) homogenizer at 10,000 rpm. To the homogenate, 25 mL of 0.11 M NaCl in 0.02 M HCl was added and the pH was measured. If needed, concentrated HCl was added dropwise to the mixture until the pH was <2. Then the mixture was homogenized for 1 min. After the layers separated, the chloroform layer, containing lipids, free fatty acids and the naphthenic acids, was removed by pipette from the bottom of the flask and 50 mL of chloroform was added to the homogenate. The mixture was homogenized for 1 min, the chloroform was removed, and another 50 mL of chloroform was added. The mixture was homogenized for 1 min and the three portions of chloroform were pooled. As a preliminary cleanup step, the combined chloroform extract was extracted with three 25-mL portions of 0.5 M aqueous Na_2CO_3 to move free fatty acids and naphthenic acids into an aqueous phase. The combined aqueous extract was acidified to pH<2 with concentrated HCl and extracted with three 20-mL portions of dichloromethane. The organic layers, containing free fatty acids and naphthenic acids, were pooled, treated with anhydrous Na_2SO_4, and taken to dryness under a flow of nitrogen.

The extract was then passed through a 500-mg SAX solid phase extraction column (Bond Elut, Varian, Missisauga, ON) for further cleanup. The cleanup method was adapted from Jones et al. (2001), and began with conditioning the column with 2 mL of hexane and loading the sample dissolved in 0.5 mL of hexane. The sample vial was rinsed with 0.2 mL of hexane and 0.2 mL of dichloromethane, followed by 0.5 mL of dichloromethane, and these three rinses were loaded onto the SAX column. The column was washed with 4 mL of hexane followed by 4 mL of dichloromethane and the residual solvent in the column was evaporated by flushing the column with air. The sample was eluted from the column with 6 mL of diethyl ether containing 2% ($v\ v^{-1}$) formic acid. The extract was taken to dryness under a flow of nitrogen, then dissolved in 0.1 mL of dichloromethane and derivatized with *N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) (St. John et al., 1998) prior to analysis by GC-MS.

Similarly, cattails collected from a process-affected wetland, Test Pond 5, were extracted for naphthenic acids. Cattail sections, 8-g samples of lower leaf, middle leaf or upper leaf, and combined rhizome and roots (Figure 1), were extracted, cleaned up, and derivatized as described above. The combined rhizome and roots from a second plant collected at Pond 5 were washed with 0.5 M aqueous Na_2CO_3 for 5 min. The aqueous Na_2CO_3 wash was filtered through a Whatman 1 filter and extracted through the same procedure as the Na_2CO_3 clean up step for the extraction of naphthenic acids from plant tissue. The roots were separated from of the washed rhizome, and approximately 8 g of roots were removed and extracted by the procedure described above. An 8-g portion of the rhizome was cut from the plant root and similarly extracted.

GC-MS method

Details of the method are given in Holowenko et al. (2002). Derivatized control cattail extracts were dissolved in 50 μL of dichloromethane, and 2 μL of this solution was injected into the GC-MS. Following method development using control cattails spiked with Merichem naphthenic acids, the GC-MS underwent maintenance to clean the instrument to improve sensitivity. Following this, cattail leaf extracts were dissolved in 100 μL of dichloromethane, and 1 μL of this solution was injected into the GC-MS. The total ion chromatograms (TICs, Murray et al., 2006) and RICs (*m/z* = 267) (Merlin et al., 2007) were recorded from each sample.

Results and Discussion

Cattails spiked with commercial naphthenic acids

A portion of the cattail leaves collected from Edmonton, Alberta was spiked with 5 μg of Merichem naphthenic acids/g of plant and extracted. A sample of leaves that was not spiked with naphthenic acids was similarly extracted and the extracts were analyzed by GC-MS. In the cattail sample spiked with naphthenic acids, a hump in the RIC for nominal *m/z* = 267 between 15 and 20 min was observed, indicating the presence of naphthenic acids (Figure 2).

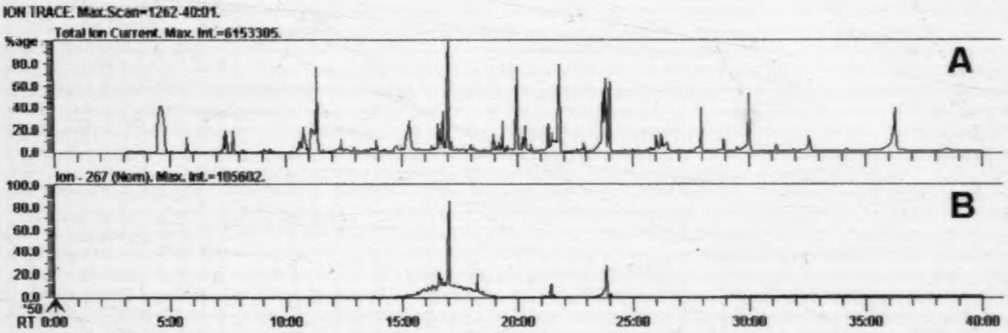


Figure 2 Total ion current chromatogram (A) of the extract of cattail leaf samples that was spiked with 5 µg of Merichem naphthenic acids /g of plat. The reconstructed ion chromatogram for the nominal *m/z* = 267 (B) shows the hump indicating the presence of naphthenic acids in the plat extract.

This hump was not observed in the RIC for nominal mass *m/z* = 267 for the cattail sample that was not spiked with naphthenic acids (Figure 3). The extraction and GC-MS method can detect naphthenic acids in cattail samples. Previous work detecting naphthenic acids in fish estimated the detection limit to be 1 µg of naphthenic acids/g of tissue (Young et al., 2006, 2007).

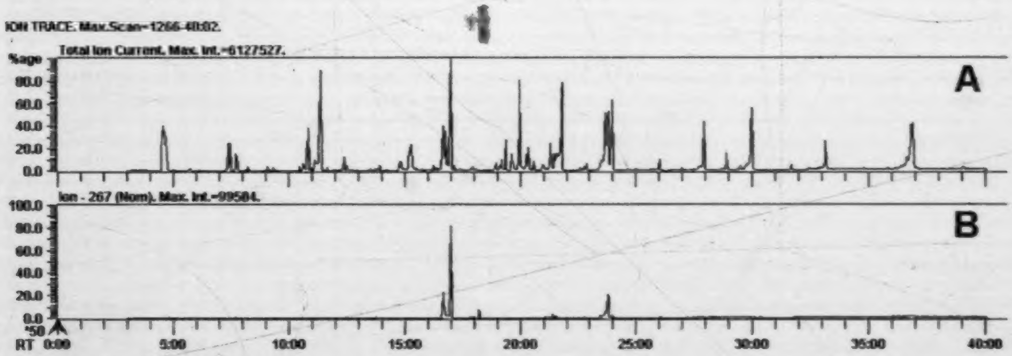


Figure 3 Total ion current chromatogram (A) of the extract of a cattail leaf sample that was not spiked with naphthenic acids and the reconstructed ion chromatogram for the nominal mass *m/z* = 267 (B).

Extraction of cattails collected from an oil sands process-affected wetland

Cattails were collected from Syncrude's Test Pond 5, a site containing approximately 24 mg naphthenic acids/L, for extraction and GC-MS analysis. A control leaf sample from a cattail collected near Edmonton, Alberta was also extracted and analyzed by GC-MS. The control leaf sample did not show the hump indicative of naphthenic acids in the RIC for nominal mass *m/z* = 267 (Figure 4). The cattails from Test Pond 5 were separated into lower leaf, middle leaf, and upper leaf, and combined rhizome and roots samples and these were extracted and analyzed by GC-MS. The leaf samples did not contain naphthenic acids (Figures 5, 6 and 7).

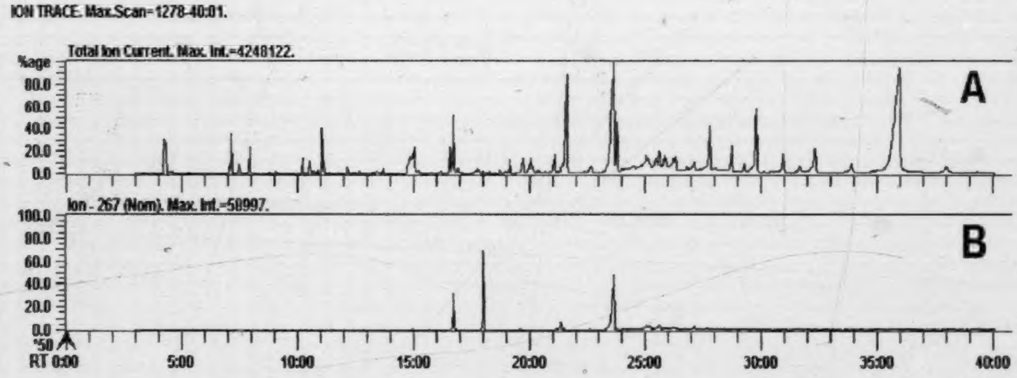


Figure 4 Total ion current chromatogram (A) of the extract of a cattail leaf sample collected from the Edmonton, Alberta area and the reconstructed ion chromatogram for nominal *m/z*=267 (B).

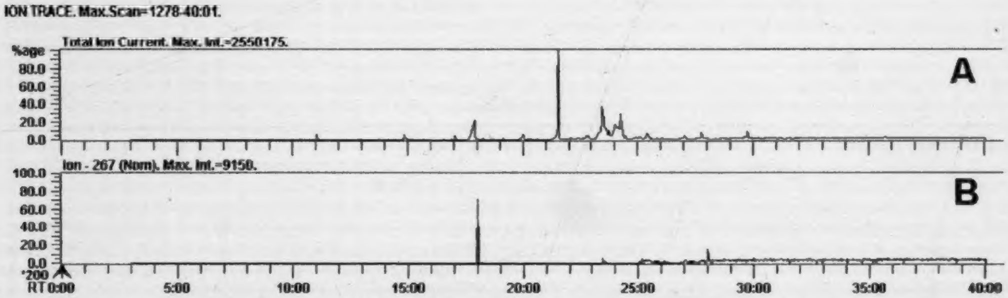


Figure 5 Total ion current chromatogram (A) of the extract of a Test Pond 5 cattail lower leaf sample and the reconstructed ion chromatogram for nominal *m/z*=267 (B).

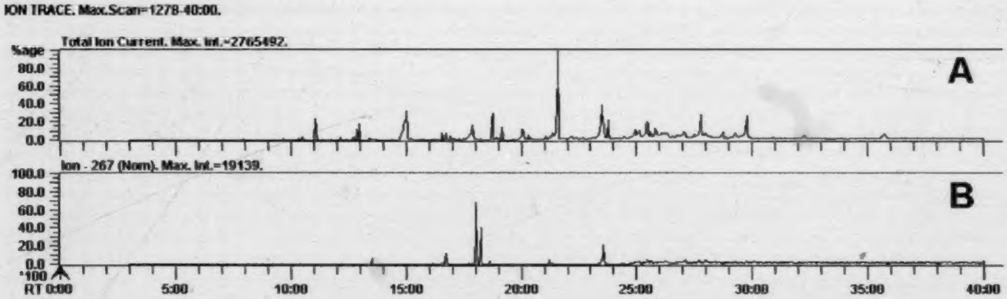


Figure 6 Total ion current chromatogram (A) of the extract of a Test Pond 5 cattail middle leaf sample and the reconstructed ion chromatogram for nominal *m/z*=267 (B).

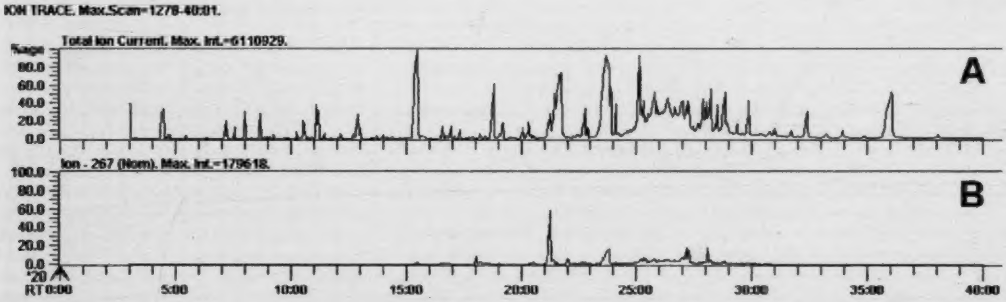


Figure 7 Total ion current chromatogram (A) of the extract of a Test Pond 5 cattail upper leaf sample and the reconstructed ion chromatogram for nominal *m/z*=267 (B).

The combined rhizome and roots of cattails collected from Test Pond 5 were extracted and analyzed by GC-MS. A control sample of cattail combined rhizome and roots collected from the Edmonton, Alberta area was also extracted and analyzed by GC-MS. The control sample did not contain naphthenic acids (Figure 8). In the extract of the Test Pond 5 combined rhizome and roots, a hump was observed in the RIC for nominal mass *m/z* = 267 between 15 and 20 min, indicating the presence of naphthenic acids (Figure 9).

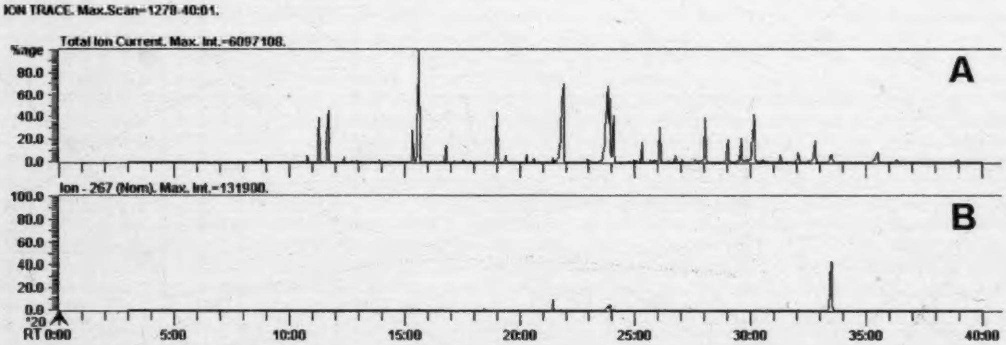


Figure 8 Total ion current chromatogram (A) of the extract of a cattail combined rhizome and roots sample collected from the Edmonton, Alberta area and the reconstructed ion chromatogram for nominal *m/z*=267 (B).

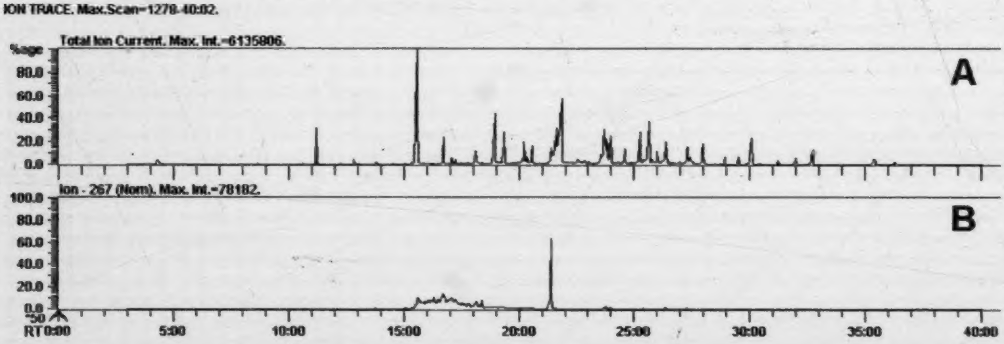


Figure 9 Total ion current chromatogram (A) of the extract of Test Pond 5 combined rhizome and roots. The reconstructed ion chromatogram for nominal *m/z*=267 (B) shows the hump indicating the presence of naphthenic acids in the extract.

c

Although naphthenic acids were detected in the Test Pond 5 combined rhizome and roots, it was unclear if the naphthenic acids were present on the surface, inside the roots or in the rhizome, which had no direct contact with the environment. A second sample of combined rhizome and roots from Pond 5 cattails was washed with 0.5 M aqueous Na_2CO_3 for 5 min to remove naphthenic acids from the surface of the combined rhizome and roots. The wash was acidified and extracted by the same procedure as the plant extracts. A hump appears in the total ion current chromatogram of the wash extract (Figure 10A) between 15 and 20 min suggesting the presence of naphthenic acids. The presence of naphthenic acids in the wash is confirmed by the hump in the RIC for m/z = 267 (Figure 10B). A hump corresponding to naphthenic acids was also observed in the RIC for m/z = 267 for the extract of the cattail roots that were washed with 0.5 M aqueous Na_2CO_3 prior to extraction and GC-MS analysis (Figure 11). A small hump was observed in the RIC for m/z = 267 for the extract of the cattail rhizome after washing, extraction, and GC-MS analysis (Figure 12), suggesting the presence of naphthenic acids inside the rhizome.

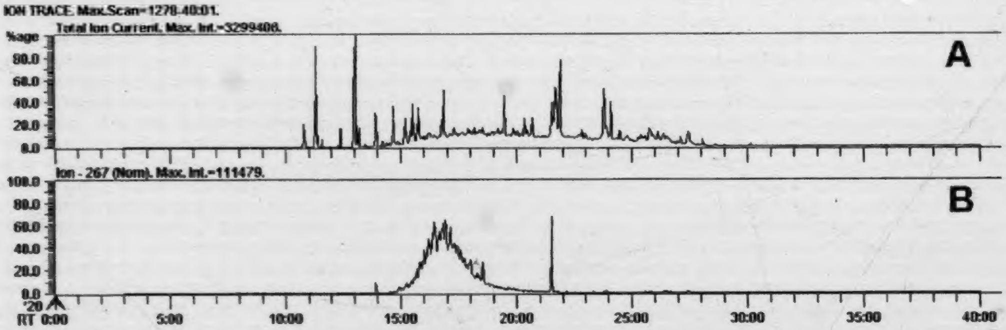


Figure 10 Total ion current chromatogram (A) of the extract of the combined rhizome and roots wash. The reconstructed ion chromatogram for nominal m/z=267 (B) shows the hump indicating the presence of naphthenic acids in the wash.

The uptake of organic compounds by plants is determined by the chemical properties of the organics. Doucette et al. (2005) observed that sulfolane, a highly water soluble sulfur compound, is transported to cattail leaf tips, whereas diisopropanolamine, a water-soluble weak base, concentrates in cattail roots. Compounds with log K_{ow} values between 0.5 and 3 can potentially move into plant cells and into cell fluids (Pilon-Smits, 2005). Naphthenic acids are a complex mixture of compounds with different carbon numbers and ring structures. Naphthenic acids with lower carbon numbers and fewer rings have log K_{ow} values closer to 2, whereas naphthenic acids with more than 12 carbons and two rings have log K_{ow} values closer to 4 (Havre et al., 2003).

Likely, only some naphthenic acids in a mixture would be capable of moving into plant roots and be transported to the leaves. The naphthenic acids with higher log K_{ow} values would be stuck in root cell walls and membranes. Further studies are necessary to determine the minimum detectable concentration of naphthenic acids in cattails.

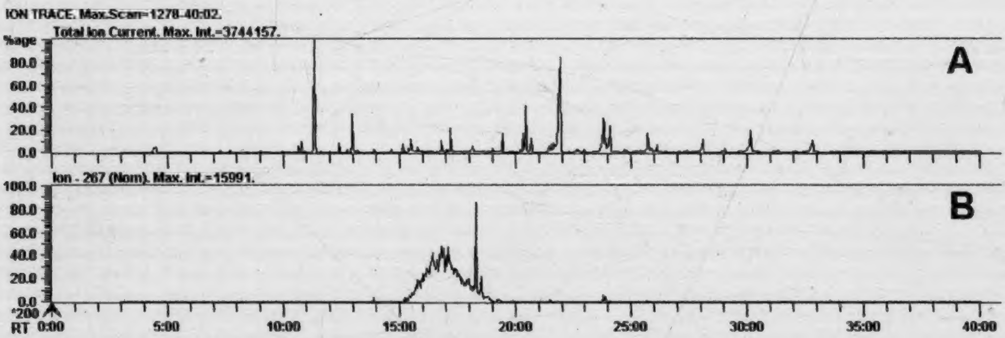


Figure 11 Total ion current chromatogram (A) of the extract of Test Pond 5 roots washed with 0.5 M aqueous Na_2CO_3 prior to extraction and GC-MS analysis. The reconstructed ion chromatogram for nominal *m/z*=267 (B) shows the hump indicating the presence of naphthenic acids in the root extract.

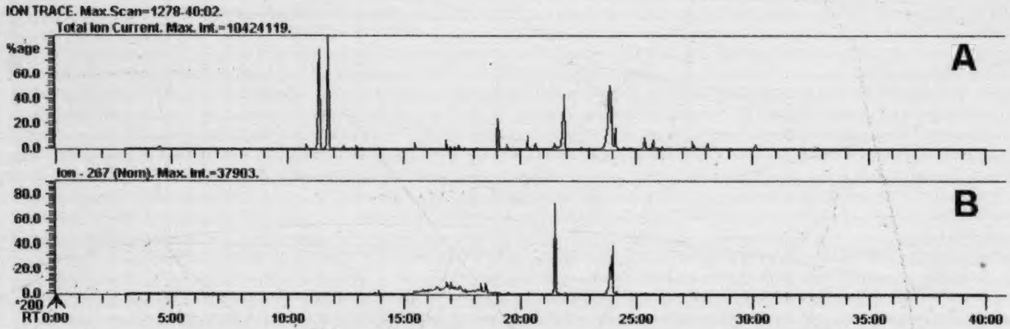


Figure 12 Total ion current chromatogram (A) of the extract of the Test Pond 5 rhizome after washing with 0.5 M aqueous Na_2CO_3 prior to extraction and GC-MS analysis. The reconstructed ion chromatogram for nominal *m/z*=267 (B) shows the hump indicating the presence of naphthenic acids in the rhizome.

Cattails from a process-affected wetland, Test Pond 5, were extracted and analyzed by GC-MS. Naphthenic acids were detected in all extracts of combined rhizome and roots from Test Pond 5 cattails. However, the washing treatment may have been too severe for the root cells. The washing treatment should be repeated using a solution closer to pH 8 rather than the 0.5 M aqueous Na_2CO_3 wash at pH 12. Distinguishing between naphthenic acids that are loosely associated with the root surface and naphthenic acids that are incorporated into root cell membranes may be a difficult problem requiring further investigation.

Literature cited

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J.J.R., Cabo, M.L., 2005. Modified method for the analysis of free fatty acids in fish. J. Agric. Food Chem. 53, 1903-1906.

Brient, J.A., Wessner, P.J., Doly, M.N., 1995. Naphthenic acids. In: Kroschwitz, J.I. (Ed.), In: Encyclopedia of Chemical Technology, 4th ed. Vol. 16. John Wiley and Sons, New York, NY, pp. 1017-1029.

Crowe, A.U., Han, B., Kermode, A.R., Bendell-Young, L.I., Plant, A.L., 2001. Effects of oil sands effluent on cattail and clover: photosynthesis and the level of stress proteins. Environ. Pollut. 113, 311-322.

Doucette, W.J., Chard, J.K., Moore, B.J., Staudt, W.J., Headley, J.V., 2005. Uptake of sulfolane and diisopropanolamine (DIPA) by cattails (*Typha latifolia*). Microchem. J. 81, 41-49.

Havre, T.E., Sjoblom, J., Vindstad, J.E., 2003. Oil/water-partitioning and interfacial behavior of naphthenic acids. J. Dispersion Sci. Technol. 24, 789-801.

Holowenko, F.M., MacKinnon, M.D., Fedorak, P.M., 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Res. 36, 2843-2855.

Jones, D.M., Watson, J.S., Meredith, W., Chen, M., Bennett, B., 2001. Determination of naphthenic acids in crude oils using nonaqueous ion exchange solid-phase extraction. Anal. Chem. 73, 703-707.

Kamaluddin, M., Zwiazek, J.J., 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. Tree Physiol. 22, 1265-1270.

MacKinnon, M.D., Boeger, H. 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Water. Pollut. Res. J. Can. 21, 496-512.

Merlin, M., Guigard, S.E., Fedorak, P.M., 2007. Detecting naphthenic acids in waters by gas chromatograph-mass spectrometry. J. Chromatogr. A. 1140, 225–229.

Merlin, M., Guigard, S.E., Fedorak, P.M., 2005. The application of a GC-MS method to detect naphthenic acids in natural waters, sera, and tissues. Progress report submitted to Albian Sands Energy Inc., Canadian Natural Resources Ltd., Suncor Energy Inc., and Syncrude Canada Ltd. October 4, 32 pp.

Murray, K.K., Boyde, R.K., Eberlin, M.N., Langley, G.J., Li, L., Naito, Y., 2006. IUPAC Standard Definitions of Terms relating to Mass Spectrometry. IUPAC project 2003-056-2-500. http://www.msterms.com/docs/IUPAC_MS_Terms_Second_Draft.pdf

Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15-39.

Siwik, P.L., Van Meer, T., MacKinnon, M.D., Paszkowski, C.A., 2000. Growth of fathead minnows in oilsand-processed wastewater in laboratory and field. Environ. Toxicol. Chem. 19, 1837-1845.

Sojda, R.S., Solberg, K.L., 1993. http://www.nwrc.usgs.gov/wdb/pub/wmh/13_4_13.pdf

St. John, W.P., Rughani, J., Green, S.A., McGinnis, G.D., 1998. Analysis and characterization of naphthenic acids by gas chromatography-electron impact mass spectrometry of *tert.*-butyldimethylsilyl derivatives. J. Chromatogr. A. 807, 241–251.

Tissot, B.P., Welte, D.H., 1978. Petroleum Formation and Occurrence. Springer-Verlag, New York, NY.

Verbeek, A.G., Mackay, W.C., MacKinnon, M.D., 1994. A toxicity assessment of oil sands wastewater: a toxic balance. Can. Tech. Rep. Fish. Aquat. Sci. 1989, 196-207.

Young, R.F., Orr, E.A., Goss, G.G., Fedorak, P.M., 2007. Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water. Chemosphere, 68: 518-527.

Young, R.F., Orr, E., Goss, G.G., Fedorak, P.M., 2006. Assessing various factors that might contribute to fish tainting by components in oil sands tailings waters. Final Report submitted to CONRAD Wetlands and Aquatic Working Group – Fish Tainting Committee. August 28, 47 pp.

Appendix B: Settings for Narrow Mass Range Scanning

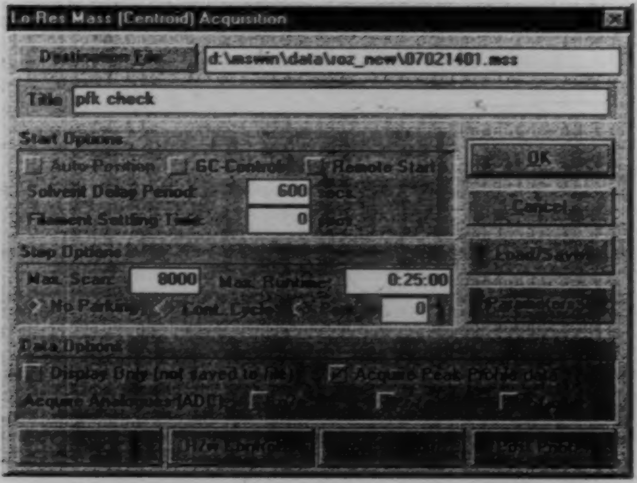
Narrow mass (270 – 265μ) scans on the VG70E

Mass calibration was accomplished using PKF as mass lock.

I. MSS set-up

Assuming a good mass calibration set up the acquisition as follows:

Profile data collection is enabled; this is optional but seems to slightly improve the signal-to-noise.


Lo-Res Mass (Centroid) Acquisition

Destination File: d:\mswin\data\voz_new\07021401.mss

Title: pfk check

Start Options

☐ Auto-Position ☐ GC-Controlled ☐ Remote Start

Solvent Delay Period: 600 secs

Filament Settling Time: 0 secs

Stop Options

Max. Scan: 8000 Max. Runtime: 0:25:00

○ No Parking ○ Cont. Cycle ○ Park 0

Data Options

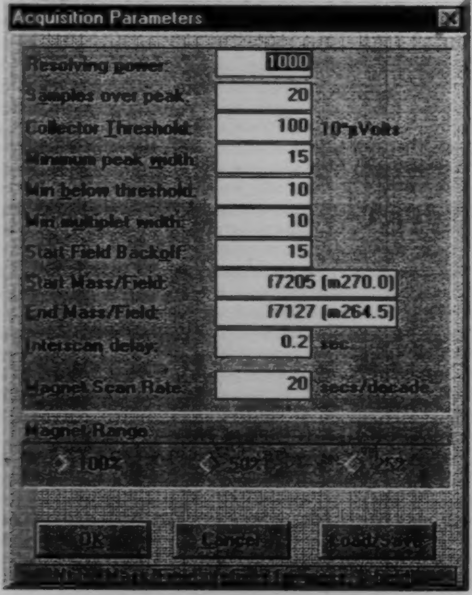
☐ Display Only (not saved to file) ☑ Acquire Peak Profile data

Acquire Analogues (ADC): ☐ ☐ ☐

These setting will approximate at 1 scan/1.5 s,

Acquisition parameters

The acquisition parameters are showing in the window below modified the bottom 5 settings. These can be loaded from "NA narrow scan profile" and the Start Field Backoff was reduced from 500. Setting the Ion window to a mass (eg 267) will increase the S/N relative to the TIC. The MS was set to acquire from 270 to 264.5.



Acquisition Parameters

Resolving power: 1000
Samples over peak: 20
Collector Threshold: 100 10*µVolts
Minimum peak width: 15
Min below threshold: 10
Min multiplet width: 10
Start Field Backoff: 15
Start Mass/Field: f7205 (m270.0)
End Mass/Field: f7127 (m264.5)
Interscan delay: 0.2 sec
Magnet Scan Rate: 20 secs/decade

Magnet Range
● 100% ○ 50% ○ 25%

II. Instrument Set-up

Gain – SEM 450, FA3 - 5 x 10^{-7} (for some reason, this gives less electronic noise than 10^{-6}). Modify these with sample concentration.

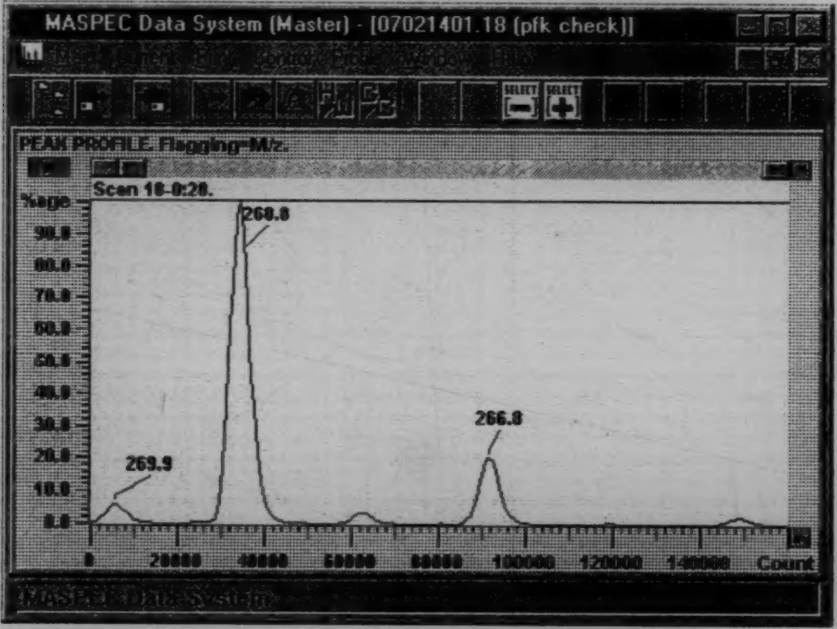
Response time – 3 (300 Hz) produces smooth, undistorted mass profiles

Scan Control / Main Scan / Mass – Low: 300 High: 328 (normal 700 – 50μ settings are ~100 and 500 respectfully). Both values may need tweaking if the magnet does not multiscan after acquisition has started.

Scan Control / Main Scan / Rate – 2 (outer dial) x 1 (inner)

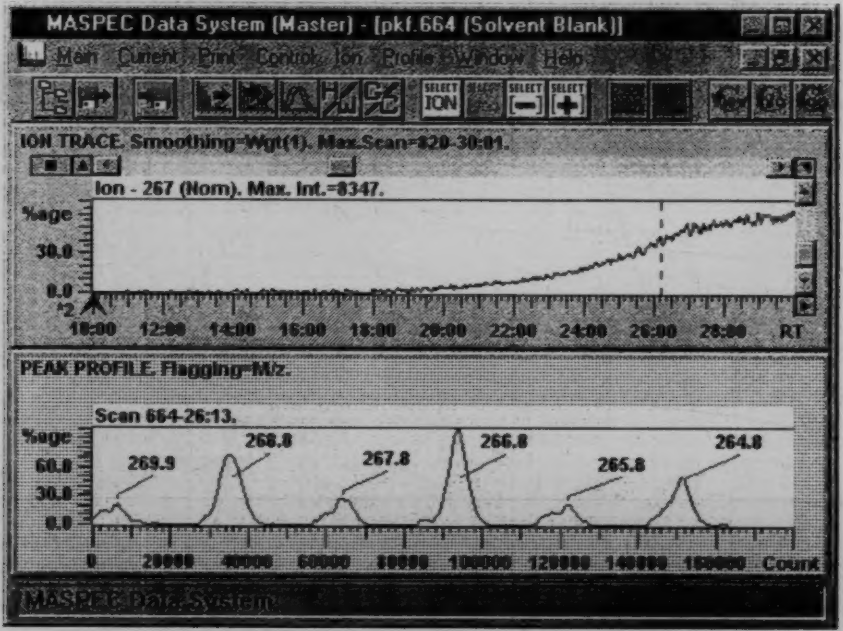
The GC split time can be increased from 0.2 seconds to 0.5 (or greater) to maximize sample loading on the column since chromatographic resolution is secondary to sensitivity.

Check the calibration with PFK and adjust the FA3 zero to give something like this:

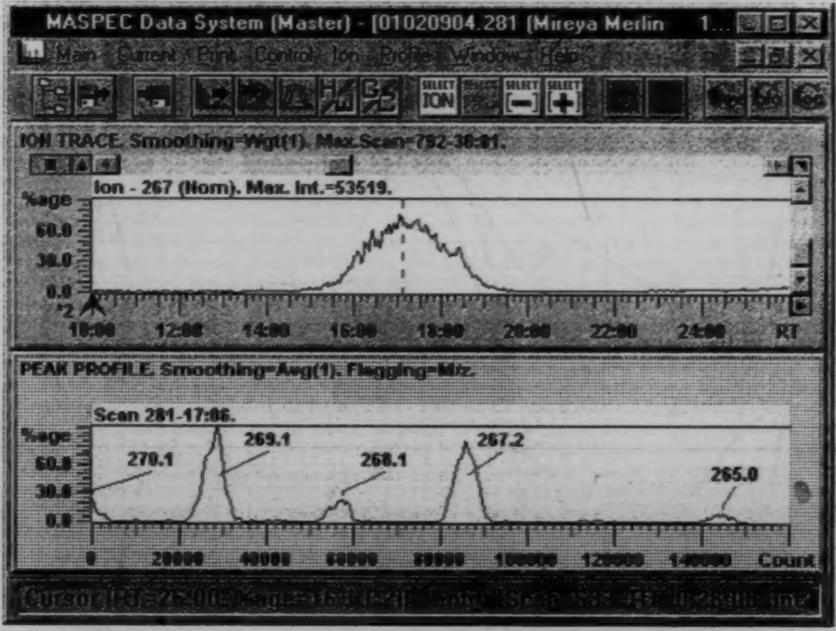

MASPEC Data System (Master) - [07021401.18 (pfk check)]
SELECT
SELECT
PEAK PROFILE Flagging=M/z.
Scan 18-0:28.
%age
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
268.8
269.9
266.8
0
20000
40000
60000
80000
100000
120000
140000
Count
MASPEC Data System

III. Acquisition

Pump out the PFK and run the samples. It will take some time for the PFK to subside to an acceptable level (269μ never disappears at high gain), so a solvent blank should be run in the GC each time after calibration. Window below showed the solvent blank (dichloromethane) in the ion -267 and the diagnostic profile window.


MASPEC Data System (Master) - [pkf.664 (Solvent Blank)]
Main Current Print Control Ion Profile Window Help
SELECT ION
SELECT
SELECT
ION TRACE. Smoothing=Wgt(1). Max.Scan=820-30:01.
Ion - 267 (Nom). Max. Int.=8347.
%age
30.0
0.0
*2
10:00
12:00
14:00
16:00
18:00
20:00
22:00
24:00
26:00
28:00
RT
PEAK PROFILE. Flagging=M/z.
Scan 664-26:13.
%age
60.0
30.0
0.0
269.9
268.8
267.8
266.8
265.8
264.8
0
20000
40000
60000
80000
100000
120000
140000
160000
Count
MASPEC Data System

Window below showed the reconstructed ion chromatogram for the nominal mass of 267 in narrow scan mode and the diagnostic profile window for an extract of Merichem naphthenic acids that is run prior to the samples to check the calibration and any shift in the retention time.



MASPEC Data System (Master) - [01020904.281 (Mireya Merlin 1...

ION TRACE. Smoothing=Wgt(1). Max.Scan=792-30:01.

Ion - 267 (Nom). Max. Int.=53519.

PEAK PROFILE. Smoothing=Avg(1). Flagging=M/z.

Scan 281-17:06.

Main
Current
Print
Control
Ion
Profile
Window
Help
SELECT ION
SELECT
SELECT
%age
60.0
30.0
0.0
*2
10:00
12:00
14:00
16:00
18:00
20:00
22:00
24:00
RT
%age
60.0
30.0
0.0
270.1
269.1
268.1
267.2
265.0
0
20000
40000
60000
80000
100000
120000
140000
Count